University of Alberta

Short- and long-term flexibility in the locomotor system of the cat

by

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Abstract

This thesis examines three forms of locomotor flexibility in the cat. The first is the timing of the swing-to-stance transition. The timing of this event varies depending on the locomotor task, and my research shows that the degree of hip flexion may be a sensory signal that initiates this event at an appropriate time.

The second form of locomotor flexibility this thesis examines is the ability of walking cats to step over obstacles. My research shows that the movement of the hindlegs over obstacles is influenced by a long-lasting and precise memory. The movement of the forelegs over the obstacle may be an essential component of initiating this memory.

The third form of locomotor flexibility that this thesis examines is a persistent increase in step height following perturbations during the swing phase of walking. This modification is only expressed in the context in which the perturbations were encountered.

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This thesis is dedicated to my parents.

"I codint of don it wethout Joe [and Joan]"

David, April 24, 1988 (age 7)

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Abbreviation list

AB (Anterior Biceps)

ANOVA (Analysis Of Variance)

CPG (Central Pattern Generator)

Dopa (DihydrOxyPhenylAlanine)

EMG (ElectroMyoGram)

IP (IlioPsoas)

LG (Lateral Gastrocnemius)

LS (Lateral Suprasylvian)

MG (Medial Gastrocnemius)

PB (Posterior Biceps)

Sart (Sartorius)

Sol (Soleus)

ST (SemiTendinosus)

VL (Vastus Lateralis)

Chapter 1[1]

1.1 Introduction

A fundamental output of the nervous system of any animal is movement. Movement is essential to meet immediate needs, such as respiration and ingestion, as well to accomplish longer-term goals such as migration, predator avoidance, and reproduction.

One fascinating form of movement is locomotion. Nearly all animals, from worms to humans, use some form of patterned muscle activation to move through their environment, whether it be air, water, or land. This ubiquity makes locomotion an important subject of study to gain insight into topics such as the development of the neural circuits during growth, the production of rhythmic neural output, and the evolution of the nervous system. A particularly important feature of locomotion is flexibility. Throughout their lives, animals increase in size, sustain injuries, and become weaker or stronger. Furthermore, they move through varied environments such as snow, sand and ice. These ever-changing requirements suggest that flexibility is an essential feature of natural locomotion, and that understanding the mechanisms underlying this flexibility is an important aspect of understanding natural locomotion. This study of flexibility in simple locomotor systems can give important insights into learning and plasticity in more complex neural systems.

The central goal of this thesis is to gain insight into flexibility in the walking system of the cat via three research projects. The first project evaluates the role of the

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position of the hip joint in triggering transitions from the swing phase to the stance phase during walking. The second project presents a unique role for long-lasting memory during steps modified using visual information. The third project describes a long-lasting and context-dependent modification of the walking pattern of the cat in response to repeated environmental perturbations. This introductory chapter contains a brief overview of the neuronal basis of locomotion, followed by a discussion of the mechanisms underlying plasticity in the locomotor system of the cat. Finally, the role of vision and memory during locomotion are presented.

1.2 The basic pattern of quadruped locomotion is produced by rhythmic neurons of the spinal cord

An obvious feature of undisturbed locomotion is its rhythmicity. Nearly a century of research has revealed that the basis for this repetitive pattern lies in the activity of rhythmically active regions of the spinal cord, termed the central pattern generator (CPG). Early support for this hypothesis came from the work of Graham Brown (1912), who showed that alternating activity persisted in flexor and extensor muscles after cutting the dorsal roots of spinalized cats to isolate the spinal cord from almost all inputs. This led to the influential concept of 'half-centres', putative regions of the spinal cord which were mutually inhibitory and which generated alternating activity in flexors and extensors. More recently, a comparison of locomotor behaviour of decerebrate walking cats with and without intact dorsal roots reported that many of the complex features of normal locomotion are not dependant on phasic sensory input (Grillner and Zangger 1984). Further evidence of intrinsically rhythmic output from the spinal cord comes from

studies of the spinal cord during fictive locomotion, a technique in which all movement is prevented either by transaction of the efferent fibers or through use of paralyzing agents (Duysens and Van de Crommert 1998). Recordings from the efferent nerves of the ventral roots shows activity alternating between antagonistic motoneurons, despite the absence of any rhythmic sensory feedback, in both chronic and acute spinal cats (Baker et al. 1984, Grillner and Zangger 1979) and decorticate cats (Perret and Cabelguen 1980).

The half-centre model alone cannot explain certain complex aspects of locomotion. For example, some flexor muscles produce multiple bursts of activity even in the absence of phasic sensory input (Grillner and Zangger 1984) and exhibit dramatically altered patterns of activity in tasks such as incline walking (Carlson-Kuhta et al. 1998, Smith et al. 1998b) and stepping over obstacles (see chapter 3 of this thesis). A refinement of the half-center model accounts for these observations by suggesting that subpopulations of neurons in the half-centers may supply rhythmic output to individual muscle groups (Grillner 1981). These subpopulations, termed 'unit-burst generators', could be combined in various ways to allow very flexible output (Smith et al. 1998a, Smith et al. 1998b).

Recently, McCrea and colleagues have reported findings that suggest that the central pattern generator may be composed of two distinct elements. They reported that when the rhythm in the flexion and extension activity in fictive locomotion breaks down, it often spontaneously returns in phase with earlier activity (Lafreniere-Roula and McCrea 2005). They propose that a timing element of the central pattern generator sends signals to a pattern shaping element, which then activates motoneurons. It is the timing element that preserves the correct rhythm even when the rhythmic output from the pattern

shaping element is absent. This proposal is compatible with both the half-center hypothesis and the unit-burst generator hypothesis.

1.3 The basic pattern of locomotor output is highly influenced by afferent feedback

A centrally generated pattern of locomotion would be insufficient to accommodate the variable terrain and changing musculo-skeletal system faced by walking animals. During the past two decades, researchers have shown the important contribution of afferent feedback in shaping the basic locomotor output to adapt to this variability. Three roles for afferent feedback have emerged from this work: determining the timing of phase transitions; influencing the amplitude of ongoing muscle activity; and adapting the locomotor pattern to long-lasting changes of the musculo-skeletal system or the environment (Pearson 2004).

1.3.1 The role of afferent feedback in determining the timing of phase transitions during walking in the cat

The feline spinal cord, isolated from descending inputs by decerebration or spinalization, can adapt the duration of the stance phase of locomotion to match the speed of a treadmill moving underfoot. This simple observation illustrates the need for an understanding of the role of afferent feedback in influencing phase transitions during walking (Pearson et al. 1998). Two early studies suggested that sensory signals related to ankle unloading (Duysens and Pearson 1980) and hip extension (Grillner and Rossignol 1978) were important in establishing the timing of phase transitions. Subsequent investigations have helped to establish the underlying neural basis for these effects.

Strong evidence has accumulated that signals from force-sensitive afferents in ankle extensors are involved in the prolongation of extensor activity described above. The locomotor pattern in both fictive (Conway et al. 1987) and decerebrate (Pearson et al. 1992) cats can be entrained to stretches applied to the ankle extensors. Furthermore, direct electrical stimulation of group I afferents from extensor muscles of the leg can prolong the stance phase of locomotion (Whelan et al. 1995). Force-sensitive Ib afferents are assumed to underlie this process, because it requires a stimulus strength that activates Ib fibers. Furthermore, small amplitude, high frequency stretches that activate Ia afferents do not have the same effect.

Researchers have also been successful at investigating the effects of hip movement on establishing the rhythm of locomotor activity. Following the observations of (Grillner and Rossignol 1978), it was reported that the fictive locomotor output of paralyzed cats could be entrained to imposed hip movements (Andersson and Grillner 1983). A later investigation revealed that muscle afferents, rather than skin or joint afferents, were likely responsible for the influence of hip motion (Kriellaars et al. 1994). Muscle spindles are the most likely afferent responsible, because there is little activity in Golgi tendon organs in fictive preparations (Pearson et al. 1998). Direct evidence for this hypothesis came from selectively activating muscle spindles of hip muscles by vibration, which advanced the onset of flexor activity in decerebrate cats (Hiebert et al. 1996).

In addition to influencing the onset of flexor activity, afferent signals related to hip flexion also influence the duration and magnitude of flexor activity. Electrical stimulation of iliopsoas and sartorius afferents at group I strength during fictive locomotion prolongs and enhances ongoing flexor activity (McCrea 2001, Perreault et al.

1995) either via mono- or disynapitic excitatory pathways (Quevedo et al. 2000) or directly via the CPG (Lam and Pearson 2001, McCrea 2001, Perreault et al. 1995). In decerebrate cats, Lam and Pearson (2001) reported that assisting the flexion of the hip in decerebrate walking cats advanced the transition from flexor to extensor activity, while resisting hip flexion had the reverse effect. The authors showed that feedback from the sartorius muscle was largely responsible for this effect both by showing the effect depended on transmission in sartorius afferents by denervating sartorius (Lam and Pearson 2001) and by replicating the effects using stimulation of sartorius afferents (Lam and Pearson 2002).

1.3.2 The role of afferent feedback in enhancing ongoing extensor activity in the walking cat

Different locomotor tasks, such as walking up or down inclines and slow and fast walking, require different amounts of propulsive force and thus different muscle activation (Donelan and Pearson 2004). A number of investigations support the hypothesis that afferent feedback related to loading in extensor muscles has an important influence on the magnitude of activity during such different tasks. One useful strategy for investigating this hypothesis has been to unexpectedly remove the support surface during walking. When cats walking on a treadmill unexpectedly step into a hole, there is a large decrease in ankle extensor activity in intact (Gorassini et al. 1994), spinalized (Hiebert et al. 1994), and decerebrate cats (Hiebert and Pearson 1999). Resisting the ankle extension into the hole (Hiebert and Pearson 1999) or stimulating group I afferents during the step into the hole (Hiebert et al. 1995) prevents this decrease. More precise results were

obtained by training cats to walk across a series of pegs instrumented with force transducers, one of which could drop suddenly. When the peg was dropped during the stance phase of locomotion, there was a drop in the measured ground-reaction force, followed by a short latency drop in ankle extensor activity. When the peg stopped and ground reaction force increased, there was a subsequent increase in the level of ankle extensor activity (Donelan and Pearson 2004). The close association between muscle activity and ground reaction force, but not muscle length or velocity, suggests the afferents underlying this effect are force sensitive Golgi tendon organs (unpublished). Experiments involving the direct manipulation of muscle afferent feedback by stretching ankle extensors (Bennett et al. 1996, De Serres et al. 2002, Stein et al. 2000) or electrical stimulation of nerves (Angel et al. 1996, Guertin et al. 1995, Pearson and Collins 1993) support this putative role for Golgi tendon organ feedback. A recent study added further support by showing the excitatory effect of stretching ankle extensors could only be due to Golgi tendon, and not muscle spindle, feedback (Donelan and Pearson 2004).

1.3.3 The role of afferent feedback during adaptive modifications of the locomotor pattern in the cat

In order to produce stable and efficient walking, the locomotor output of walking animals is very flexible (Pearson 2004). A well-studied example of adaptation to a musculoskeletal change is the recovery of normal kinematics of walking following the denervation or weakening of muscles in the leg of the cat (Bouyer et al. 2001, Carrier et al. 1997, Gritsenko et al. 2001, Misiaszek and Pearson 2002, Pearson et al. 1999). In all cases, initial locomotor deficits caused by the weakened muscles were overcome by

compensatory adaptations in other muscles. A second example is that cats adapt their motor output when stepping over obstacles to compensate for a weight attached to the shank of the leg (Lam & Pearson, unpublished). Walking animals also adapt to persistent changes in the environment. Intact walking cats decrease the latency of the flexion response after repeatedly stepping in a hole (Gorassini et al. 1994), for example, and they alter the pattern of flexion to compensate for a repeated upward movement of the walking surface (McVea et al. 2004). There is also clear evidence of such adaptive responses in humans, who modify their locomotor output to compensate while walking on split-belt treamills (Jensen et al. 1998), rotating disks (Jurgens et al. 1999, Weber et al. 1998) and a sled that moves unexpectedly (Reynolds and Bronstein 2003).

One particular adaptive modification that has been studied in a number of preparations is the response to steps that are repeatedly obstructed during the swing phase of locomotion. When walking cats contact an obstacle during the swing phase of locomotion, they exhibit a consistent pattern of ankle extension and knee flexion that lifts the paw up and over the obstacle (Buford and Smith 1993, Forssberg 1979, Wand et al. 1980) called the stumbling-corrective response. Study of this reflex in chronic spinal animals (Forssberg et al. 1975, Forssberg et al. 1977) as well as in decerebrate fictive locomotion (Quevedo et al. 2005a, Quevedo et al. 2005b) suggests the basic pattern of this response is organized at the spinal cord, although the response is much more robust in intact cats (see chapter 4 of this thesis). Ordinarily, the pattern of walking returns to normal following a single stumbling-corrective response (see chapter 4 of this thesis). However, animals have been shown to respond to repeated stumbling-corrective response by increasing the height of the step to avoid the obstructing obstacle. This occurs in both

decerebrate (Lou and Bloedel 1988a, Lou and Bloedel 1988b) and spinal (Edgerton et al. 2004) animals. This increase in step height persists following the removal of the obstacle for a small number (1-10) of steps.

Determining which neural structures underlie adaptive modifications of locomotor output is the subject of ongoing study (Edgerton et al. 2004, Pearson 2001, Pearson 2004, Rossignol et al. 2004). Changes during adaptive such modifications are believed to include alterations of the networks generating rhythmic locomotor output (Carrier et al. 1997, Pearson et al. 1999), changes in signals descending from supra-spinal regions (Carrier et al. 1997), and increases/decreases in the strength of locomotor reflexes (Fouad and Pearson 1997, Pearson et al. 1999). Although the *site(s)* of these adaptive responses are uncertain, research suggests that afferent feedback is an important signal during the *establishment* of these adaptations (Pearson 2004). One compelling example of this is that the adaptive responses to muscle denervation described above do not occur if afferent feedback from the muscles involved is eliminated, either by chemical ablation (Pearson et al. 2003) or immobilization (Pearson and Misiaszek 2000). Furthermore, successful recovery from spinal injury in cats requires use (de Leon et al. 1998) and this improvement is not attributable to changes in muscle properties resulting from use (Rossignol 2000). Finally, preliminary data suggests that the adaptation to a repetitive movement of the walking surface does not occur in cats with reduced muscle afferent feedback (McVea et al. 2004). In humans as well there is good evidence that adaptive locomotor responses depend on afferent feedback generated during walking. Increasing or decreasing load-related feedback (by supporting or weighting the walking subject) increases and decreases, respectively, the adaptive response to walking on a split-belt

treadmill (Jensen et al. 1998). In addition, adaptive responses to walking on rotating disks do not generalize to movement in a wheelchair (Gordon et al. 1995), they do not require vestibular feedback (Earhart et al. 2004), and they do not result from a sensory conflict between visual and afferent feedback (Jurgens et al. 1999). All of these findings have been interpreted as evidence that the adaptive responses under study rely heavily on afferent feedback from the moving limbs.

1.4 Neurobiology of visually-guided modification of stepping in the cat

One important modification of stepping that does not rely on feedback from muscle afferents is the visual guidance of steps. It is the role of supraspinal areas to modify the basic rhythmic output of the spinal cord to make anticipatory adjustments based on visual information about the external world. There are two primary pathways through which this is done (Drew et al. 2002). The first is located *medially* within the spinal cord, and includes the vestibulo- and the reticulo-spinal pathways. The vestibulospinal neurons originate in the lateral vestibular nucleus, where they receive inputs signaling the orientation and movements of the head from the vestibular apparatus of the inner ear. The reticulospinal neurons originate in the reticular formation of the brainstem, a diffuse area involved in the control of basic behaviours such as breathing, walking, and sleeping. Both vestibulo- and reticulospinal neurons are active during locomotion, although the latter play a more important role in visually-guided gait modifications. The second major descending pathway is located in the *lateral* section of the spinal cord, and this chapter will focus on the corticospinal neurons of this pathway, which originate primarily in the motor and premotor cortices. The lateral and medial

descending pathways have different but overlapping roles in the visual control of locomotion, which are discussed below.

1.4.1 Descending signals from the motor cortex are important during visually-guided stepping

Many descending inputs carried to the spinal cord via corticospinal neurons of the lateral pathway originate in the motor cortex. Consequently, understanding the role of these signals in visually guided locomotion has relied heavily on recordings from, or manipulation of, the motor cortex of the cat. During normal walking, it seems that the motor cortex has no essential role, as lesioning (Adkins et al. 1971) or inactivating it (Beloozerova and Sirota 1993), or sectioning the medullary pyramids (Liddell and Phillips 1944) (through which the corticospinal tract passes) have no lasting effect on walking over flat surfaces (Armstrong and Marple-Horvat 1996). However, many studies have shown that neurons of the motor cortex are rhythmically active during such walking (Armstrong and Drew 1984, Drew et al. 2002, Widajewicz et al. 1994) and that stimulating the motor cortex artificially (Armstrong and Drew 1985) results in increased muscle activity and subsequent movement in the legs. Therefore, while the motor cortex may not be essential during normal walking, its rhythmic activity may be involved in processes such as transitioning between the stance and swing phase of locomotion (Armstrong and Drew 1984, Drew et al. 2002) and modifying the strength of cutaneous reflexes (Bretzner and Drew 2005). There is no question, however, that the motor cortex has an essential role in locomotion that is visually guided, such as landing the paw precisely or modifying the step to avoid an obstacle. Cats that have had the output of the motor cortex disrupted by sectioning the medullary pyramids are unable to step

accurately on ladder rungs (Liddell and Phillips 1944) and knock over obstacles in their path following lesioning of the motor cortex (Beloozerova and Sirota 1993). When output from the motor cortex which normally results in hindleg flexion is disrupted, either by lesioning the medial descending pathways to the forelegs, or temporarily inactivating parts of the motor cortex, the hindlegs do not successfully step over obstacles (Drew et al. 1996, Drew et al. 2002). These findings are confirmed by recordings from the motor cortex that show the majority of neurons display increased activity when cats step on ladder rungs (Beloozerova and Sirota 1993) or over obstacles (Drew 1993, Widajewicz et al. 1994). Interestingly, when stepping over obstacles, some cells of the motor cortex are active *between* the time that the foreleg and the hindleg steps over the obstacle (about 800 ms), and these cells typically were responsive to sensory input from both the hindlimb and the forelimb. These cells have been suggested to coordinate the hind- and forelegs when stepping over obstacles (Widajewicz et al. 1994) (although proprioceptive pathways and subcorticals structures may also be involved (Drew et al. 2004)).

1.4.2 Motor cortical signals are integrated into spinal networks to influence movements around primarily one joint

One interesting question which follows from these observations is, "What purpose does the activity in the motor cortex serve?" One possibility would be that the signals from neurons of the motor cortex encode a 'high level' signal, such as the path of the foot over an obstacle, which could be achieved by multiple patterns of joint movement and muscle activity. Another possibility is that the activity of neurons within the motor cortex represents a 'low-level' signal which could only be expressed in one way, such as the temporal pattern of muscle activation that will lift the limb. Although the nature of the

signal encoded in the motor cortex is a subject of vigorous debate (Georgopoulos and Ashe 2000, Scott 2000, Scott 2003) it seems likely that in the case of visually guided leg movements in the cat, the motor cortex encodes a low-level signal (Drew et al. 2004). A close comparison of the activity in the flexor muscles that are active while stepping over obstacles with activity of individual cells of the motor cortex shows that activity in different cells are closely correlated with particular muscles, and less so with others. This correlation includes time of initiation, duration of activity, as well as intensity of activity (represented by firing frequency in cortical neurons and level of activation for flexor muscles) (Drew et al. 2002). However, it is important to emphasize that there is not a direct linkage between signals descending from the motor cortex and flexors of the legs. Corticospinal neurons in the cat synapse onto interneurons, and not motoneurons. These interneurons are modulated by other interneurons as well as propriospinal neurons (Kalaska and Drew 1993). Drew and colleagues have proposed a scheme in which the output of cortical neurons integrates into different networks of interneurons, each of which produces rhythmic movements at one joint during walking (these are called unit burst generators after (Grillner 1982)). Output from cortical neurons would primarily affect the unit burst generators of one joint, but could also influence neighboring joints via axon collaterals (Drew et al. 2004). This scheme fits with the experimental observations described above, and suggests a method for the differential movement of joints without disrupting the basic pattern of locomotion (Drew et al. 2004).

1.4.3 Signals descending via reticulospinal tracts help maintain posture and balance during visually-guided stepping

We now return briefly to the reticulospinal tract, the other major descending pathway important for visually guided stepping. When walking animals step over obstacles, the required leg movements have the potential to disrupt balance and lead to instability (Drew et al. 2004). As a result, the limbs that remain on the ground must make compensatory actions to keep the animal stable and to propel it up over the obstacle (Lavoie et al. 1995). The reticulospinal tract is believed to control these postural adjustments. Stimulation of the areas of the brainstem associated with reticulospinal neurons produces diffuse changes in locomotion and overall posture (Drew and Rossignol 1984, Mori et al. 1992), and cats with damage to the medial tracts of the spinal cord, but not the lateral tracts, have impaired posture and weigh support which varies in severity depending on the extent of damage to reticulospinal tracts (Brustein and Rossignol 1998). Furthermore, reticulospinal neurons receive inputs from the same cells of the motor cortex that send signals to the spinal cord via corticospinal neurons (Kably and Drew 1998), which provides them with the information necessary to make compensatory postural adjustments (Drew et al. 2004).

More direct evidence for the postural role of reticulospinal neurons during visually-guided stepping comes from recordings of the activity of these neurons while cats step over obstacles. Like corticospinal neurons, the majority of reticulospinal neurons show increases in their activity as the legs pass over obstacles (Prentice and Drew 2001). This pattern of activity is complex, and cells often show multiple peaks of activity throughout the modified step. This complex activity may reflect the fact that reticulospinal neurons receive inputs from multiple regions of the motor cortex, including

regions related to two or three limbs (Drew et al. 2004, Prentice and Drew 2001). Unlike the simpler output of corticospinal neurons, which influence primarily the pattern of movement around one joint as cats make visually-guided steps, the output of reticulospinal neurons seems to represent a pattern of postural adjustments needed across multiple joints or even multiple limbs. Spinal interneurons are then responsible for integrating this pattern into ongoing movements such that adjustments are made in appropriate muscles at appropriate times (Drew et al. 2004). This is consistent with studies that show that activity in the reticular formation and reticulospinal neurons can modify the activity of multiple groups of flexors or extensors, depending on the phase of locomotion when the activity occurs (Drew and Rossignol 1984, Drew et al. 1986).

1.4.4 Cells in lateral suprasylvian cortex, responsive to optic flow, may process important visual information during locomotion

It is not clear which regions of the brain are involved in the avoidance of obstacles during locomotion. One important visual signal used to locate objects during locomotion is believed to be the direction and speed of movement of visual stimuli across the retina (called *optic flow*) (Gibson 1958, Sherk and Fowler 2001). In the cat, the lateral suprasylvian (LS) cortex, an area which receives inputs from the retina via the superior colliculi and the lateral posterior nucleus of the thalamus (Rauschecker et al. 1987), has been shown to be responsive to optic flow in a fashion which would be relevant to movement generated during locomotion. Cells in this region are especially responsive to stimuli moving outwards on the retina, away from the center of gaze. This sort of stimuli would be generated by an observer approaching an obstacle, and (Rauschecker et al.

1987) suggest cells of this region may be involved in fixating and detecting objects during locomotion. In addition, cells of LS have been shown to be receptive to stimuli in both visual hemispheres and to respond particularly well when stimuli presented to the two eyes move in opposite directions, a pattern of optic flow which would be present when approaching a close-up obstacle (Sherk and Fowler 2001). Finally, cats that have had area LS ablated have less accurate foot placement and make more errors when walking among obstacles (Sherk and Fowler 2002), which suggests output from this region is important when making visually guided steps. Other subcortical areas, such as the basal ganglia, have also been suggested as sources of visual input to the motor cortex (Drew et al. 1996).

1.5 Summary

Although the basic pattern of walking is generated by the spinal cord, this pattern is heavily influenced by feedback from muscle afferents, inputs from cutaneous afferents, and descending commands from the cortex related to visual input. These inputs can lead to both short-term, step-to-step modifications as well as long-term modifications of locomotor output. This thesis attempts to gain insight into the mechanisms underlying some examples of flexibility in the walking system of the cat.

In the second chapter, I examine the role of the position of the hip during the transition from the swing phase to the stance phase of locomotion. The main finding of this study is that the degree of flexion of the hip influences the initiation of stance in both decerebrate and intact cats.

In the third chapter, I test the role of memory in guiding the hindlegs of cats over obstacles. The main finding of this study is that the movement of the hindlegs is controlled by a long-lasting and precise memory of the obstacle. Additional data presented in the discussion suggests that the movement of the forelegs over obstacles is an important component of the establishment of this memory.

In the fourth chapter, I describe a form of adaptive plasticity in the cat in response to repeated perturbations during the swing phase of walking. This plasticity is uniquely long-lasting, context dependent, and depends on the cortex to be established.

These findings are relevant to many fields of neuroscience. Study 2, for example, has interesting implications for the study of working memory during natural movement (see Chapter 3). All three studies are particularly interesting to those who study locomotion. Flexibility is an integral feature of locomotor systems and must be accounted for in any complete description of locomotion (Pearson 2004). Furthermore, recovery from injury to the locomotor system may rely on this plasticity (Edgerton et al. 2004, Pearson 2001) making a solid understanding of adaptive modifications of walking essential for clinical neuroscience.

1.6 References

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in the four animals. For example, the ankle angle was always smallest when the leg was leading over an object, and largest when walking on the treadmill (Figure 2.6).

We also examined the angular velocity of the hip joint, and found that it varied up to five-fold from trial to trial at the time of the swing-to-stance transition, even within the same task (data not shown). Furthermore, the maximum angular velocity of the hip occurred up to 300 ms before the onset of extensor activity.

Although our observations on joint angles indirectly support the hypothesis that afferent signals related to hip position play a role in initiating extensor activity, another possibility is that a signal derived from a combination of information from sensory receptors distributed throughout the leg is a critical factor regulating the onset of extensor activity. For example, this signal could provide information about global variables such as the position of the paw relative to the body or to the ground. The former has been termed 'limb axis' by Bosco and Poppele (2001). To examine whether these variables could provide a reliable signal for initiating the swing to stance transition, we calculated the length and angle of the limb axis at the time of extensor activation (we define limb axis as the angle between the toe, the hip, and a vector from the hip, forward and parallel to the walking surface) as well as the distance and direction of the toe from where it eventually contacted the ground for the different tasks.

Figure 2.7 compares the variability of the limb axis *angle* to the variability of the hip, knee, and ankle angles at the time of the onset of MG activity for all tasks. In all four animals, the angle of the limb axis was more variable than the hip angle but less variable than the knee or ankle angles. We also examined the variability of these angles at the time of onset of MG activity within tasks (data not shown). The variability of all angles

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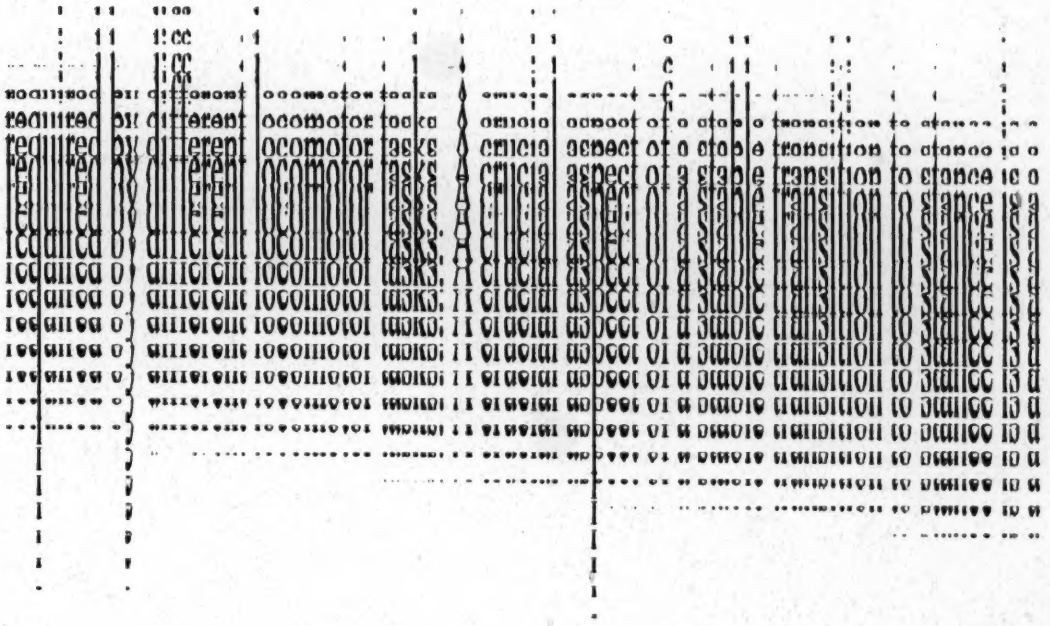
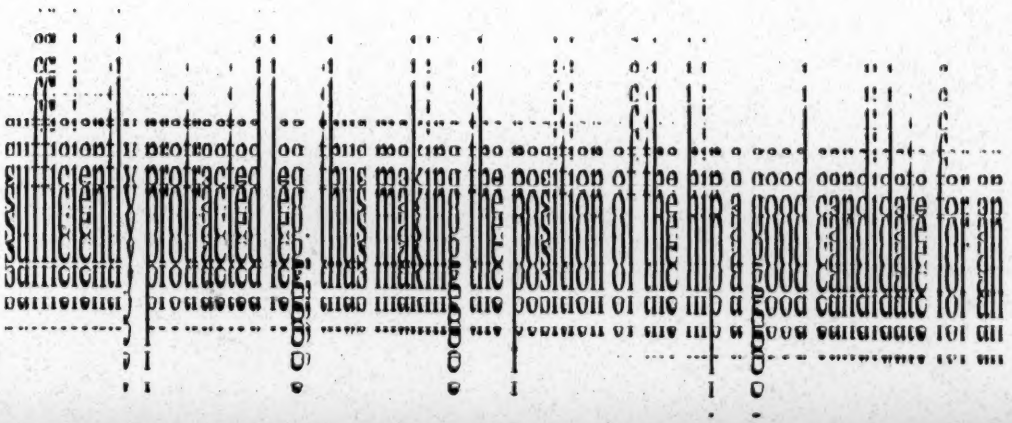
ensure correct placement of the foot during stance, despite the different movements





ensure correct placement of the foot during stance, despite the different movements required by different locomotor tasks. A crucial aspect of a stable transition to stance is a sufficiently protracted leg, thus making the position of the hip a good candidate for an afferent signal regulating the swing-to-stance transition. Evidence for this role has come from recent observations of the effects of perturbing hip movements in decerebrate walking cats (Lam and Pearson 2001). Assisting hip flexion during the swing phase results in a shortening of flexor burst duration and an increase in duration of the subsequent bursts in extensors. Resisting hip flexion during swing has the opposite effect. In some situations resisting hip flexion leads to the maintenance of flexor activity for the duration of the resisting perturbation (Lam and Pearson 2001). Another indication for a role of hip afferents in promoting the transition from flexion to extension is that imposed flexion movements of the hip during fictive locomotion in DOPA/nialamide treated spinal cats shorten flexor burst duration when applied near the end of the flexor bursts (Andersson and Grillner 1981). Recently, a similar observation has been made in the foreleg during fictive locomotion in decerebrate cats (Saltiel and Rossignol 2004). Protraction of the shoulder near the end of the burst activity in flexor motoneurons shortens the duration of flexor bursts and promotes an earlier onset of extensor activity. Thus in both hind legs and forelegs there are good indications that sensory receptors in proximal regions of the legs are involved in controlling the timing from swing to stance.

An important aspect of this transition in the hind legs is the initiation of activity in knee and ankle extensor muscles commencing about 80ms before ground contact. This pre-stance extensor activity occurs during the first extension (E1) phase of the locomotor cycle, i.e. during the period when the knee and ankle joints are extending (Engberg and

Lundberg 1969). Thus gaining an understanding of the mechanisms regulating the timing of swing-to-stance transition requires knowledge of the neuronal mechanisms responsible for initiating burst activity in the knee and ankle extensor muscles near the end of the swing phase. As mentioned earlier, there is now some evidence from reduced preparations to suggest that afferent signals linked to flexion movement of the hip joint can influence the timing of the onset of extensor activity. If this is also true for normal walking animals then we predicted that some feature(s) of the kinematics of movement at the hip joint should be correlated with the timing of the onset of the E1-associated extensor activity. A similar logic was used in an earlier study reporting a role for hip afferents in regulating the extension to flexion (stance-to-swing) transition (Grillner and Rossignol 1978). Thus one objective of this investigation was to determine the relationship between joint kinematics and the time of onset of ankle extensor activity in normal walking cats. To dissociate movements at the hip from movements at the knee and ankle joints, we examined animals stepping in a variety of situations: a horizontal treadmill, up and down steps, and stepping over objects.

Another objective of the present investigation was to examine in more detail the influence of imposing flexion movements of the hip on extensor activity in decerebrate walking cats. This influence was examined only qualitatively in the earlier study (Lam and Pearson 2001). Here we determined the relationship between the time of termination of flexor bursts and the time of onset of the subsequent extensor bursts with the aim of establishing the extent to which these two events are linked when hip flexion is assisted. In addition we measured the value of hip joint angle at the time of the transition from flexor to extensor activity with and without assisting hip flexion during the swing phase

to assess whether a sensory signal related to hip position could be a factor in the initiation of the swing-to-stance transition. A preliminary description of some of our findings has been published (Pearson et al. 2003a).

2.2 Materials and methods

Two sets of experiments were performed in this investigation. The first was designed to examine the influence of assisting hip flexion during walking in decerebrate walking cats and the second was designed to investigate the relationship between electromyographic (EMG) activity in ankle extensor and hip flexor muscles and the kinematics of hind leg movement in normal walking cats. All experimental procedures were approved by the Health Sciences Animal Welfare Committee at the University of Alberta.

2.2.1 Assisting hip flexion in decerebrate walking cats

The procedure for examining the effects of assisting hip flexion was similar to that used in an earlier investigation from our laboratory (Lam and Pearson 2001). Briefly, each animal (n=2) was anesthetized with Isoflurane and a tracheal cannula inserted for continued administration of the anesthetic. Blood pressure was monitored via a cannula inserted into one carotid artery and the other carotid was ligated. One jugular vein was cannulated for the administration of fluids and drugs. The left hind leg was then partially dennervated by cutting the saphenous, sural, superficial peroneal, and distal tibial nerves. This removed cutaneous input from most of the hind leg. Bipolar recording electrodes (Cooner wire AS632) were then sewn into the iliopsoas (IP) and medial gastrocnemius (MG) muscles of both hind legs. The wires of these electrodes were led under to skin to a

multi-terminal connector positioned above the animal's back. Next, the iliac crests were exposed and a stout wire was threaded through holes drilled in both crests. The two ends of the wire were clamped to each crest. This wire was later clamped to an external frame to support the hindquarters while the animal was walking on a treadmill. Reflective markers (diameter about 0.5 cm) were placed above the iliac crest, the hip joint, knee and ankle joints, and on the paw and toe of the left leg. These markers were used to determine the kinematics of leg movements using the Peak Motus 8.2 motion analysis system (Peak Performance Technologies). Triangulation was used to determine the position of the knee joint.

Following this preparatory procedure, the animal was transferred to a frame mounted above a treadmill. The head was placed in a sterotaxic holder and the wire through the iliac crest fixed to a supporting frame. Approximately two and a half centimeter wide surgical tape was wrapped around the thigh of the left hind leg, and a loop of 1.5 mm string was attached to the anterior edge of the tape. This loop was used to manually assist hip flexion during walking sequences. The animal was then decerebrated by transecting the brainstem rostral to the superior colliculus and mammillary bodies and removed from the anesthetic immediately following decerebration. Both animals began to walk spontaneously about 30 minutes later, although electrical stimulation of the mesencephalic locomotor region (Shik et al. 1966; Grillner and Shik 1973) was used to facilitate walking in one animal.

2.2.2 Electromyographic and kinematic analysis of hind leg movements in intact animals

The second objective of this investigation was to determine which kinematic parameters of hind leg movement correlate with the onset of ankle extensor activity

immediately preceding the swing-to-stance transition. This was examined in four intact adult cats walking in a variety of situations: 1) a horizontal treadmill at different speeds (described as 'treadmill' in figures) 2) along a series of steps at -25, 0, and 25 degree angles (described as 'down pegs', 'level pegs', and 'up pegs' in figures), and 3) stepping over an object placed on a horizontal walkway (described as 'leading' or 'trailing' in figures). Figure 2.4 shows three of these tasks. Each animal was first trained daily for one to two weeks to walk consistently in the three situations. Training consisted of inducing animals to participate in the various tasks with food and affection rewards, and lasted for up to one hour per day. Next, bipolar EMG electrodes (Cooner wire AS632) were implanted into muscles of the right hind leg under general anesthetic (Isoflurane) and aseptic conditions. In all animals EMG electrodes were placed in the knee extensor vastus lateralis (VL), the hip flexor iliopsoas (IP), and the ankle extensors soleus (Sol), lateral gastrocnemius (LG), medial gastrocnemius (MG). In three animals electrodes were also placed in knee flexor/hip extensor semitendinosus (ST) muscle. The leads from the EMG electrodes were led under the skin to a multi-pin socket fixed with screws and dental acrylic to the animal's skull. While anesthetized, adhesive reflective markers (diameter 0.5 cm) were placed over the iliac crest, the hip, knee and ankle joints, and on the end of the paw and the fifth digit of the right hind leg.

2.2.3 Data collection and analysis

In decerebrate cats, data were collected for the duration of time that an animal walked regularly (between one and two hours). In intact cats, data were collected for approximately ten to twenty trials per day for one to two tasks, rotating through the various tasks over the course of one to two weeks. Because we were seeking kinematic

parameters which were related to the onset of stance in different types of movements, we did not screen trials based on speed or range of movement. We did screen trials qualitatively for smoothness of movement.

During regular sequences of walking in the decerebrate animals, and in intact animals walking under different conditions, the EMG signals were recorded on an 8 channel Vetter 4000A PCM recorder. One channel of the recorder was reserved for a signal from the Peak Motus motion analysis system for later use in synchronizing the EMGs with video data. During all trials video data were recorded, and a time code and a signal for synchronizing EMG with video were added to the video data. The Peak Motus system was later used to track joint movements and calculate the kinematics of movements at the hip, knee and ankle joints. The length and velocity of MG was calculated from the knee and ankle angles. The length when both knee and ankle joints were at 90 degrees was taken as zero, and changes from this were calculated using trigonometry assuming that the proximal attachment of MG is located on the femur 0.5 cm from the knee joint and the length of the attachment of MG on the calcanium is 1.5 cm from the ankle joint (Goslow et al. 1973). The velocity of the length changes was calculated by numerically differentiating the calculated length.

Following the storage of EMG and video data, the EMGs recorded from decerebrate walking animals were digitized off-line at 1 kHz using the Axotape data acquisition system (Axon Instruments). The Peak Motus system was used to track the movements of the joints and calculate the kinematics of the hip, knee, and ankle. Custom written software (Matlab) was used to measure cycle periods, burst durations, the relative timing of the onset of burst activity in different muscles, and the relationship between

muscle activity onset and kinematics. Figure 2.5 shows kinematic data and ankle extensor activity (MG muscle) from a 'down pegs' and a 'leading' trial. The angles measured at the time of onset of MG activity are indicated by the dotted lines.

The EMG data from intact animals was digitized (600 or 1200 Hz) using Peak Motus. Custom written software in Matlab was used to rectify and low-pass filter (first-order Butterworth, 20 Hz) raw EMG signals, as well as examine the relationship between kinematics and the initiation of muscle activity.

2.2.4 Statistical analysis

A two-tailed F-test, which tests for equality of variance, was used to compare the amount of variance among angles at the time of ankle extensor activation (Woolson and Clarke 2002). Linear regression was used to calculate the correlation coefficients between variables. Student's t-test was used to test for changes in cycle periods and burst durations. ANOVA test was used to compare hip angles in assisted and unassisted conditions.

2.3 Results

In normal cats walking on a horizontal surface, the onset of stance (i.e. foot contact with supporting surface) in a hind leg is preceded by extension at the knee and ankle joints. These extension movements are associated with activity in the extensors across the knee and ankle joints (Engberg and Lundberg 1969) and this activity is an early neuro-muscular event underlying the transition from swing to stance. The concept we wished to examine in this investigation was whether sensory feedback via afferents

arising in the hip region contributes to the initiation of burst activity in the ankle and knee extensor muscles.

2.3.1 Influence of imposed hip flexion during walking in decerebrate cats

A previous study from this laboratory (Lam and Pearson 2001) reported that assisting the flexion movement of the hip during treadmill walking in decerebrate cats shortened the duration of bursts in the hip flexor muscle iliopsoas (IP). In that study, the shortening of the duration of hip flexor activity in response to hip flexion was associated with an advanced onset of burst activity in the ankle extensor muscle medial gastrocnemius (MG). In the present investigation our initial goal was to examine the relationship between the time by which MG burst activity was advanced and the time by which IP burst activity was shortened.

Figure 2.1A shows electromyographic (EMG) data for a short period of walking during which flexion of the hip was assisted during one step cycle. These data clearly show that assisting hip flexion reduced the duration of the burst in the IP muscle (a2 compared to a1) and advanced the following burst of activity in the MG muscle, which is consistent with previous observations. To quantify these and similar data we plotted the reduction in the IP burst duration relative to the duration of the immediately preceding IP burst ($a_1 - a_2$ in Figure 2.1A) versus the reduction in the interval between the onset of IP bursts and the onset of the following MG burst ($b_1 - b_2$ in Figure 2.1A). Plots for two animals are shown in Figure 2.1B,C. The reduction in IP duration and the advance in MG burst onset parameters were strongly correlated ($r^2 = 0.80$ and 0.83) and the best fitting lines were close to the line of identity (dotted in Figure 2.1B,C). It is important to note that the time of the onset of the IP bursts of the assisted cycles relative to the time of

onset of the preceding IP bursts was not influenced by the imposed hip flexion (Figures 2.3A and 2.4B,C), thus eliminating the possibility that the hip flexion simply shortened the IP bursts but did not influence the time of onset of MG activity. The shortening of the IP bursts and advance of the MG bursts did not significantly influence the timing of stepping in the contralateral leg. This can be seen in the example in Figure 2.1, and was quantified by comparing the change in cycle period of the contralateral leg with the change in IP burst duration (not shown). Changes in the contralateral cycle period were insignificant ($p > 0.05$, paired t-test) and not correlated to changes in the IP burst duration ($r^2 = 0.015$ and 0.070 for the two animals.) There were also no significant changes in the duration of the contralateral IP burst, indicating the absence of any influence on the timing of the contralateral swing-to-stance transition.

In addition to reducing the duration of the IP bursts, the imposed hip flexions also reduced the amplitude of these bursts (Figure 2.2). This can be seen in the example shown in Figure 2.2A and in the comparisons of the averages of the IP bursts with and without imposed hip flexion (Figure 2.2B,C). The shaded regions in the top traces in Figures 2.2B,C indicate the average effect of assisting hip flexion in two animals. Note also in these figures that the timing of onset of the IP bursts relative to the preceding IP burst was similar during unassisted (top traces) and assisted (bottom traces) flexion of the hip.

The next issue we explored was the relationship between the hip angle at the time of the transition from flexor (IP) to extensor (MG) activity. Figure 2.3A shows records of the hip angle and IP activity for a short walking sequence during which hip flexion was assisted for one cycle. Note the clear reduction in the duration and amplitude of the IP

bursts produced by the imposed movement, and that the hip angle at the times of the termination of the IP burst was similar during the assisted cycle (indicated by the arrow on the plot of hip angle) and unassisted cycles. The lower ends of the bar graphs in Figure 2.3B,C show the average of the hip angle at the time of termination of the IP bursts for assisted and unassisted cycles in two animals. In one animal (left graphs) the hip angle at the time of termination of the IP bursts was not significantly altered when hip flexion was assisted (83° with both unassisted and assisted cycles; $p = 0.133$, one-way ANOVA), while in the second animal the hip angle at the time of termination of the IP bursts was reduced by about 5° (109° with unassisted cycles and 104° with assisted cycles; $p = 0.0025$, one-way ANOVA). A noteworthy difference in the stepping behavior of the two animals was that the magnitude and rate of hip flexion was much larger in the first (Figure 2.3D,E). Furthermore, the assisted movements in the second animal noticeably increased the magnitude of hip flexion, and the change in angular velocity produced by the perturbation was larger in this animal (compare slopes of flexion movements in Figure 2.3D,E).

2.3.2 Relationships between leg kinematics and the onset of the ankle extensor activity in normal walking cats

Based on our observations in decerebrate walking cats, as well as results from previous studies (Lam and Pearson 2001; Lam and Pearson 2002a), we formed the hypothesis that signals related to hip position during swing contribute to initiating the transition from swing to stance. The issue we next explored was whether this is also true in normal walking cats. Initially we attempted to assist hip flexion in cats walking

normally on a treadmill to establish whether this perturbation advanced the onset of extensor activity. However this strategy failed because walking was consistently disrupted in an unpredictable manner thus preventing a clear assessment of the responses to the imposed flexion movements. As an alternative we examined a variety of kinematic parameters of hind leg movement when animals walked in different situations (Figure 2.4 and 5) and looked for parameters that were most closely correlated with the onset of extensor activity during the swing phase. We predicted that one of these parameters would be the hip angle.

Consistent with this prediction was our finding that the angle at the hip at the time of MG burst onset remained relatively constant in all the tasks in three of the four animals (Figure 2.6). In the fourth animal the hip angle at the onset of MG activity was similar in five of the six tasks. In contrast to the relative constancy of hip angle at the onset of MG activity, the knee and ankle angles at the same instant varied considerably depending on the task. The highest variation was seen for the ankle, which had a range of 50-60 degrees, twice that of the hip. This is apparent in Figure 2.5, which shows two example trials for one cat. The hip angle at the time of MG onset is similar for all four steps, while the knee and ankle angles vary by about 40 degrees. This suggested that the position of the hip was an important signal for the initiation of MG activity. However, it should be emphasized that this does not indicate that the position of the hip was the sole afferent signal related to this initiation. The differences between the angles of the hip at the time of MG onset during different tasks suggests that other signals, in addition to signals from the hip, are probably involved in terminating swing. The pattern of variation in the knee and ankle angles at the time of onset of MG activity for the different tasks was consistent

in the four animals. For example, the ankle angle was always smallest when the leg was leading over an object, and largest when walking on the treadmill (Figure 2.6).

We also examined the angular velocity of the hip joint, and found that it varied up to five-fold from trial to trial at the time of the swing-to-stance transition, even within the same task (data not shown). Furthermore, the maximum angular velocity of the hip occurred up to 300 ms before the onset of extensor activity.

Although our observations on joint angles indirectly support the hypothesis that afferent signals related to hip position play a role in initiating extensor activity, another possibility is that a signal derived from a combination of information from sensory receptors distributed throughout the leg is a critical factor regulating the onset of extensor activity. For example, this signal could provide information about global variables such as the position of the paw relative to the body or to the ground. The former has been termed 'limb axis' by Bosco and Poppele (2001). To examine whether these variables could provide a reliable signal for initiating the swing to stance transition, we calculated the length and angle of the limb axis at the time of extensor activation (we define limb axis as the angle between the toe, the hip, and a vector from the hip, forward and parallel to the walking surface) as well as the distance and direction of the toe from where it eventually contacted the ground for the different tasks.

Figure 2.7 compares the variability of the limb axis *angle* to the variability of the hip, knee, and ankle angles at the time of the onset of MG activity for all tasks. In all four animals, the angle of the limb axis was more variable than the hip angle but less variable than the knee or ankle angles. We also examined the variability of these angles at the time of onset of MG activity within tasks (data not shown). The variability of all angles

was lower for any individual task than that for all tasks pooled. Importantly, the difference between variability of the hip angle for individual tasks and for all tasks pooled was generally much less than the difference between the variability of individual and pooled tasks for other angles. This is consistent with our hypothesis that signals related to the hip angle contribute more to regulating the swing to stance transition than the signals related to the other three angles. Examination of the *length* of the limb at the time of onset of MG activity revealed a large variability between tasks and major differences in the profiles of the limb axis length for the different tasks (Figure 2.8). The limb axis length ranged from about 15 to 20 cms at the time of the onset of MG activity, and during steps down pegs, for example, the limb axis length generally started quite short and became longer but during leading steps over obstacles the limb axis started long and shortened dramatically through the swing phase. We also examined the position of the toe relative to where it eventually touched the ground at the time of onset of MG activity. Again, there was no consistent pattern in the distance or the angle between the toe and the point where the toe eventually touched ground and the time MG became active. This can be seen in Figure 2.6, in which the distance from the toe at the time of MG burst onset to the position of ground contact varied depending on the task.

Another sensory signal that might be used to initiate activity in the ankle and knee extensors is from the extensor muscles themselves. To examine this possibility in more detail, we calculated the profiles of length and velocity during the swing phase from the kinematics of knee and ankle movements (see Methods) in an attempt to identify a consistent relationship between the time of onset of MG activity and the length and lengthening velocity of the MG muscle. The first observation was that MG length at the

time of MG burst onset varied considerably from task to task (Figure 2.9). Assuming that gamma drive to muscle spindles is similar from task to task, this observation makes it unlikely that signals related only to muscle length are involved in initiating the onset of MG bursts. Another observation was the absence of any consistent relationship between the latency from the time of peak lengthening velocity to MG burst onset and the maximum lengthening velocity (Figure 2.10). We did observe a stronger inverse relationship when animals were walking on the treadmill at different speeds. However, the slope of this relationship was steep and the latencies at the slower walking speeds were quite large (approximately 300 ms).

A final observation of relevance to the issue of the mechanism(s) regulating the onset of extensor activity was that there was a qualitative difference in timing and duration of the IP bursts in intact animals compared to decerebrate walking animals. In decerebate animals, the termination of IP activity was always tightly coupled to the onset of MG activity (Figure 2.1), whereas in intact animals, this was not always the case depending on the task (Figure 2.11). In some situations IP was observed to be active during the E1 phase, and thus active at the same time as the E1-related activity in MG. When this occurred there was very often a clear distinction between F and E1 related activity in IP as shown in Figure 2.11B,C, with the termination of the F component of the IP bursts closely linked to the onset of MG activity (dotted lines in Figure 2.11). The relevance of these observations to our understanding of the swing-to-stance transition is considered in the Discussion.

2.4 Discussion

Recent studies in the cat have indicated that the timing of the swing-to-stance transitions in the hind legs and forelegs are regulated to some extent by proprioceptive signals from the hip and shoulder regions, respectively (Lam and Pearson 2001; Lam and Pearson 2002a; Saltiel and Rossignol, 2004). *A priori,* sensory feedback related to hip position might be particularly important in regulating swing to stance transitions in the hind legs under a variety of conditions for two reasons. First, during the swing phase of walking, the position of the hip is most reflective of the protraction of the leg, whereas the positions of the knee and ankle joints are more reflective of the height of the foot relative to the ground. Second, the movement of the hip during locomotion is biphasic, passing through any one position only once during the swing phase. In contrast, the joints of the knee and ankle both extend and flex during the swing phase, and thus may pass through the same position twice during the swing phase. The findings of the present investigation provide additional evidence that afferent signals arising from receptors in the hip region are an important component of the mechanism that regulates the swing-to-stance transition.

Although the position of the hip is a good candidate for an afferent signal regulating swing-to-stance transitions, other signals are also involved. For example, our laboratory has reported inhibitory coupling between, firstly, the systems that generate flexor activity of the two hindlegs (Lam & Pearson, 2001), and secondly, the systems that generate flexor activity of ipsilateral fore- and hind-legs (McVea et al, 2005). Furthermore, studies of fictive locomotion have shown that a range of afferents, such as

group I afferents from ankle and knee extensors, as well as group II afferents from hip and knee flexors, have an effect on the duration of flexor activity (McCrea, 2001).

2.4.1 Hip flexion influences swing-stance transition in decerebrate walking cats

Our conclusion that hip afferents have a role in regulating the timing of the swing-to-stance transition is strongly supported from our observations in decerebrate walking cats. First, assisting flexion movements of the hip joint shortened the duration of bursts in the IP muscle (hip flexor) and promoted an earlier onset of activity in the MG muscle (ankle extensor) that closely matched the shortening of hip flexor activity (Figure 2.1). Since the onset of burst activity in ankle and knee extensors shortly before ground contact is the first neuromuscular event in the swing-to-stance transition, this observation demonstrates directly that signals related to hip position could be involved in initiating this transition. Assuming that the basic network for the timing of activity in the central pattern generator (CPG) is mutual inhibition between flexor and extensor half-centers (Lundberg 1980), the simplest explanation for the changes in timing of the IP and MG bursts is that sensory signals generated during hip flexion act to terminate activity in the flexor half-center and release the extensor half-center from inhibition. Alternatively, changes in timing of the IP and MG bursts could be produced by reflex modification of interneuronal networks located between the CPG and motoneurons, or even by direct reflex actions on motoneurons. However, we believe these alternative possibilities are less likely because of the strong correlation between the time of termination of the IP bursts and the time of onset of the MG bursts (Figure 2.1B,C). Moreover, no studies on non-walking preparations have reported any reflex action from hip muscle afferents on ankle extensor motoneurons. On the other hand, the group Ia afferents from hip extensors

are known to form inhibitory connections onto IP motoneurons (Eccles and Lundberg 1958), so it is quite conceivable that increased activity in these afferents partially explains the reduction in the magnitude of the IP bursts we observed when hip flexion was assisted (Figure 2.2).

An analysis of the kinematics of the hip joint at the time of extensor activation and flexor burst termination showed the hip angle could be similar in both control and assisted step cycles (Figure 2.3). In one animal, the hip angles at the time of the termination of the IP bursts were virtually identical in the two situations (Figure 2.3B), whereas in the second animal it was decreased by about 5° during assisted trials (Figure 2.3C). We have no explanation for this difference, but it may be due to differences in the strength of hip flexion movements in the two animals (compare Figures 2.3D and E). In the first animal these movements were larger in magnitude and closer to those in normal walking animals. If this animal reflects the situation during normal walking, then the fact that hip angle at the time of the termination of IP activity remained constant suggests that a sensory signal related to hip position is involved in initiating the transition from flexor to extensor activity.

2.4.2 Hip position is part of a multi-modal signal controlling stance

Following our results from decerebrate walking animals, we predicted that the position of the hip could be an important signal controlling the swing-to-stance transition in intact walking cats. By examining the kinematics of leg movements in a variety of situations, (Figures 2.4 and 2.5) we found the hip angle at the time of onset of activity in ankle extensor muscles was relatively constant compared to knee and ankle angles

(Figure 2.6). Although indirect, we interpret this observation as evidence to support the hypothesis. It is important to note that we have not concluded that the position of the hip is the sole signal which triggers a transition from swing to stance. In fact, our data shows this to be unlikely for three reasons. Firstly, the hip angle at which MG activity begins varies somewhat from task to task. This suggests the strength of the feedback from hip-related afferents is either modulated by descending, task-dependant connections, or that other afferent signals are involved. Secondly, the hip angles at which MG became active varied from cat to cat. Thirdly, cat 4 had a more varied hip position at the time of MG onset than the other cats. These points suggest that, although the position of the hip is an important part of a multi-modal afferent signal which triggers the transition to stance, the relative contribution of hip position to this signal likely varies from cat to cat and from task to task.

Accepting that multi-modal sensory signals are important in regulating the swing-to-stance transition, with signals from the hip being especially important, we need to consider which receptors give rise to these signals. Receptors in the hip joint capsule are unlikely to be involved because inactivation of these receptors has no effect on entrainment of the fictive locomotor rhythm in decerebrate cats (Kriellaars et al. 1994). More likely possibilities are stretch sensitive receptors in hip extensor muscles and/or muscle spindles and Golgi tendon organs in hip flexor muscles (Perrault et al. 1995; Lam and Pearson 2002a). As for receptors in other regions of the leg, we know that electrical stimulation of group I afferents arising from spindles and Golgi tendon organs in ankle extensor muscles can reset the fictive rhythm to extension (Conway et al. 1987; Guertin et al. 1995) and thus have the potential for regulating the swing-to-stance transition.

However, if we assume that gamma drive is similar during the different tasks examined, we can use the length and velocity of the MG muscle as an approximate indication of from group Ia afferent activity at the time of the swing-to-stance transition. The large variation in the length of MG at the time of activation (Figure 2.9), and the weak relationship between the interval from the time of maximum MG velocity to MG activation and the maximum MG velocity (Figure 2.10), indicate that signals from receptors in the ankle extensors are unlikely to have a significant role in controlling the swing-to-stance transition in intact walking cats.

Another possible signal regulating the timing of the swing-to-stance transition is one derived from a combination of information from sensory receptors distributed throughout the leg to indicate global variables such as the position and angle of the paw relative to the hip (Bosco and Poppele 2001). However, we found that the distance between the toe and the body at the time of ankle extensor activation varied widely depending on the task (Figure 2.8) and that the orientation of the toe relative to the body was less consistent than the angle of the hip (Figure 2.7). We cannot definitively say that the endpoint of the limb is not a factor in activating extensors in late swing, but if it is, then the computation of the endpoint would necessarily require information about the position of all the joints of the leg. Our data, particularly those from decerebrate animals, show that the position of the hip would be an important component in this computation.

2.4.3 Integration of hip position signals into the CPG

An important question raised by our results is how sensory signals related to the hip position might be integrated into the CPG to influence transitions from flexor to

extensor half-centers. This requires an understanding of the functional organization of the CPG. At issue is whether or not the onset of activity in the knee and ankle extensors is linked to an overall switch from flexor to extensor activity in muscles throughout the leg. Lundberg (1980) has argued that it is not. This conclusion was based on the fact that the EMG recordings of Engberg and Lundberg (1969) showed that IP activity lasted well into the E1 phase and overlaped the early activity in ankle extensors. Lundberg concluded that "the E1 burst of activity in extensors has an origin extraneous to that of the half-centres", and proposed that the half-centers switch from flexor to extensor activity at the time of the termination of IP activity, i.e. at a time close to the time of ground contact. However, another interpretation of the same data, and one consistent with observations we have made in this investigation, is that the overall switch from flexor to extensor activity occurs at the time of onset of extensor activity, and that the E1-associated activity in IP is generated by the extensor half-center. Evidence supporting this interpretation is that the termination of IP bursts was normally associated with the onset of MG activity at or near the F-E1 transition when animals walked on the treadmill (Figure 2.11A) and, in situations in which activity in IP did occur during the E1 phase, that this activity was often distinctly segregated from the preceding flexion associated activity (Figure 2.11B,C). If the extensor-associated activity in IP is produced via an excitatory pathway linking the extensor half-center to the IP motoneurons, then we must assume that transmission in this pathway is task-dependant to allow for the variable occurrence of IP activity during E1 phase (Figure 2.11). This scheme accounts for the strong linkage between the termination of IP bursts on the onset of MG bursts that occurs in decerebrate walking animals (Figure 2.3) as a complete closing of the pathway from the extensor

half-center to IP motoneurons due to loss of supraspinal facilitation of the pathway. If this scheme is accepted, then we propose that signals generated by flexion of the hip during swing inhibit the interneuronal networks generating the F phase of flexion (flexor half-centre) and help promote the switching from the flexor to extensor half-centre. This transition is associated with the onset of activity of knee and ankle extensors, and extensor related IP activity may or may not continue depending on the state of the putative connection from the extensor half-centre to the IP motoneurons described above.

2.4.4 Summary

In this study, we have shown that the position of the hip is an important part of the signal that initiates the swing-to-stance transition in the hindlegs of the walking cat. This expands our understanding of the role of sensory feedback in regulating phase transitions during walking, and complements our knowledge of the role of hip position in regulating the stance-to-swing transition (Grillner and Rossignol, 1978; Hiebert et al. 1996). In this role, the position of the hip is one part of a multi-modal signal which initiates swing (Pearson, 2003) and we suggest that the hip has a similar role as part of a distributed signal initiating stance.

2.5 Figures

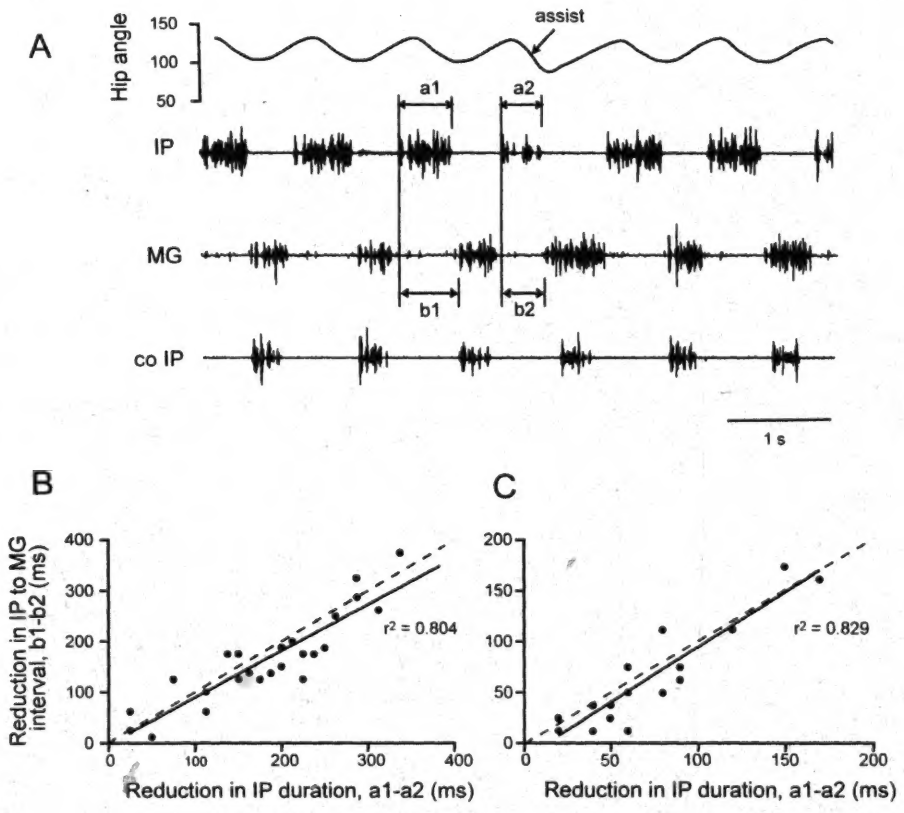


Figure 2.1

Assisting hip flexion in decerebrate walking cats reduces the duration of IP bursts and advances the onset of activity in MG. **A.** EMG recordings showing that parameters measured to quantify the changes in timing of the IP and MG bursts. During this sequence, the flexion movement of the hip during one step cycle (labeled 'assist') reduced the duration of the IP burst (a2 compared to a1) and advanced the onset of the following MG burst (b2 compared to b1). **B-C.** Scatter plots for data from two animals showing that the advance in the time of onset of the MG bursts (b1-b2) produced by assisting hip flexion is highly correlated with the reduction in IP burst duration (a1-a2). The solid lines are the best fitting lines to the data, and the dotted lines are the expected relations if the advance in the onset of MG bursts exactly equaled that reduction of the duration of IP bursts.

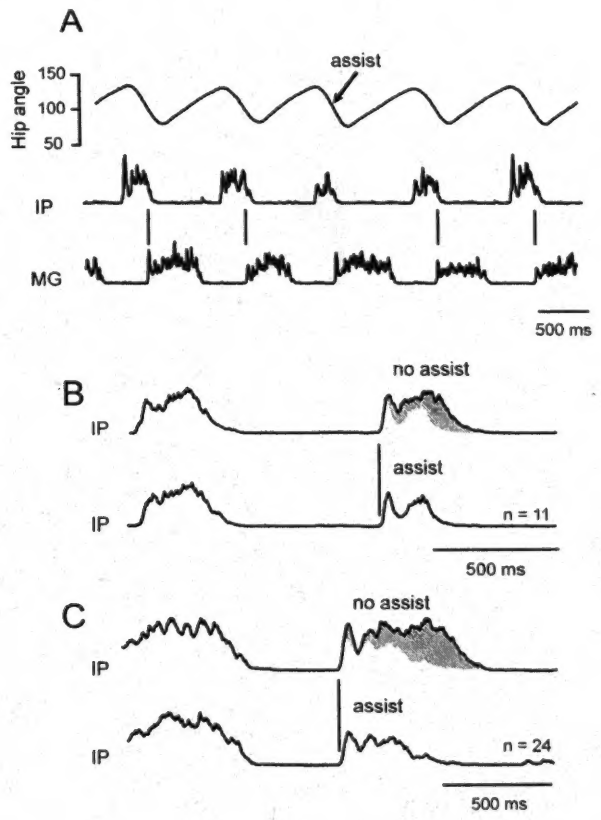


Figure 2.2

Assisting hip flexion in decerebrate walking cats reduces the magnitude of IP bursts. **A.** Rectified and filtered EMG records from IP and MG muscles during a short walking sequence showing that assisting hip flexion on one cycle (labeled 'assist') reduced the magnitude and duration of the IP. These records were from a different animal than those shown in Figure 2.1A. Note also the advance in the time of onset of MG activity associated with the reduction in IP burst duration (gray region) and the close linkage of the termination of IP bursts with the onset of MG bursts (vertical lines). **B-C.** Averages from two animals of the rectified and filtered IP bursts during unassisted (top traces) and assisted (bottom traces) flexions of the hip. The shaded regions in the top traces show the difference between the two sets of averages. Note that hip flexion decreased in magnitude and duration of the IP bursts. Note also that assisting hip flexion had no influence on the timing of the onset of IP bursts relative to the preceding IP bursts. The vertical lines indicate the reference times for the averages.

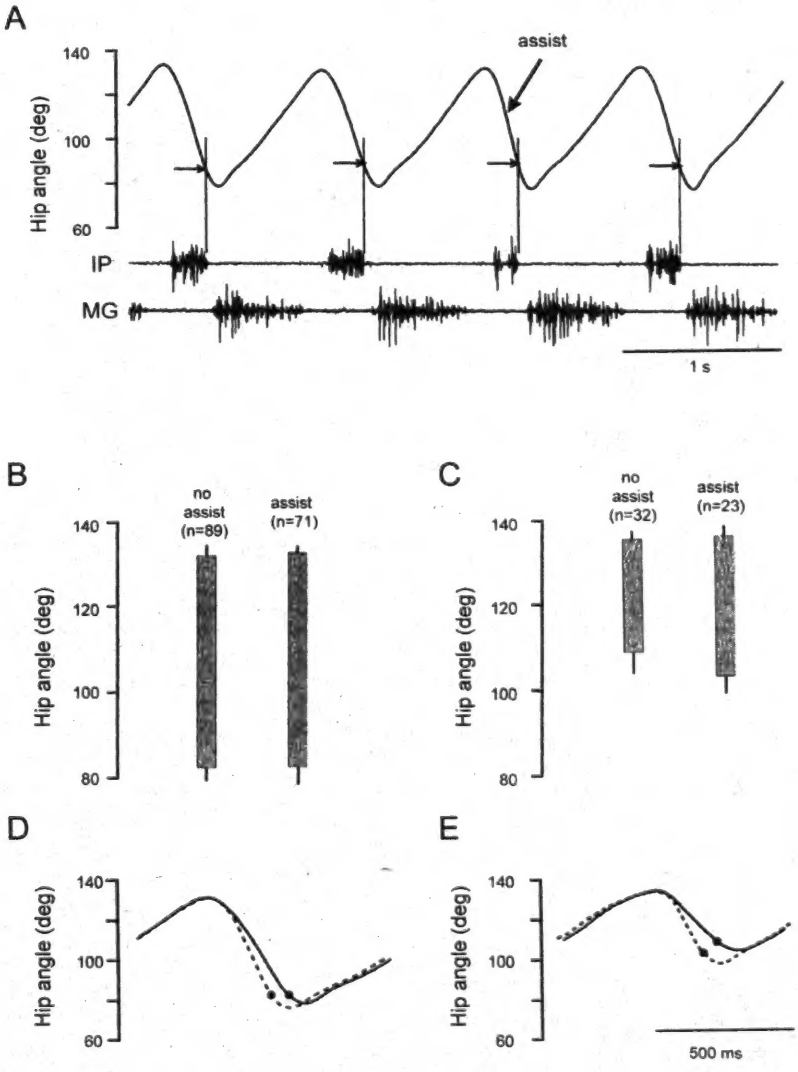


Figure 2.3

Hip angle at the time of the transition from flexor (IP) to extensor (MG) activity is similar during assisted and unassisted cycles in decerebrate walking cats. **A.** Short sequence of stepping showing a plot of the hip angle (top trace) and EMG records from IP and MG (bottom traces) during three unassisted and one assisted (labeled 'assist') cycles. The horizontal arrows indicate the hip angle at the time of termination of the IP bursts. **B-C.** Bar graphs for two animals showing the range of the hip flexion from the time of swing onset (top) to the time of termination of IP bursts (bottom). Short vertical lines indicate standard deviations. **D-E.** Superimposed plots of hip angle during one unassisted (solid lines) and one assisted (dotted lines) cycle illustrating the influence of assisting hip flexion on hip movement in the two animals yielding the data in **B** and **C**, respectively. The solid dots indicate the times the IP bursts terminated in these trials.

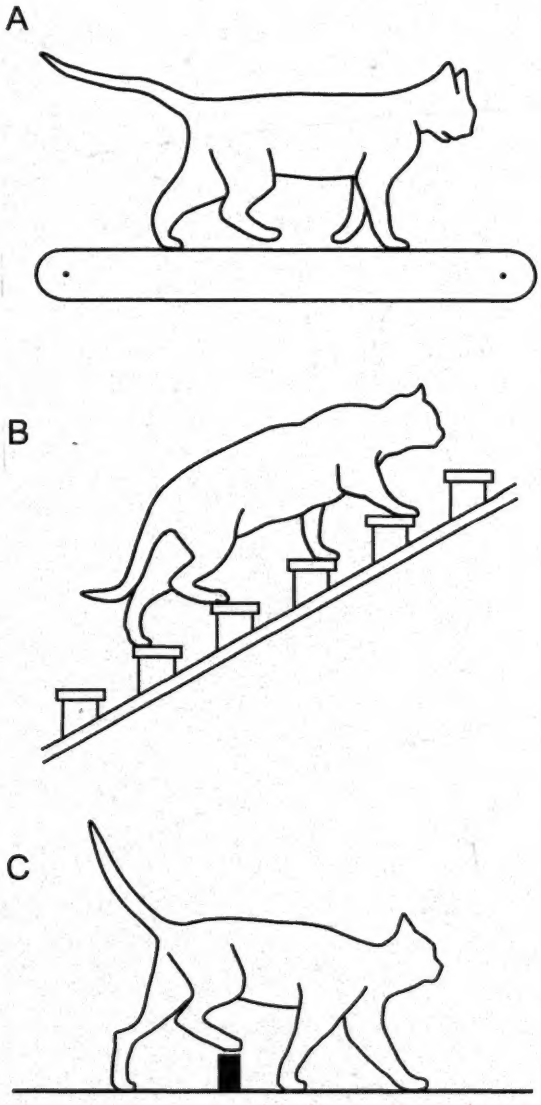


Figure 2.4

Locomotor tasks used to examine the kinematics of leg movement during the swing phase in intact walking cats. **A.** Walking on a horizontal treadmill. **B.** Walking on pegs. The slope of the pegs was either up (as shown), horizontal, or down. **C.** Stepping over blocks. The experimental leg could either lead (as shown) or trail.

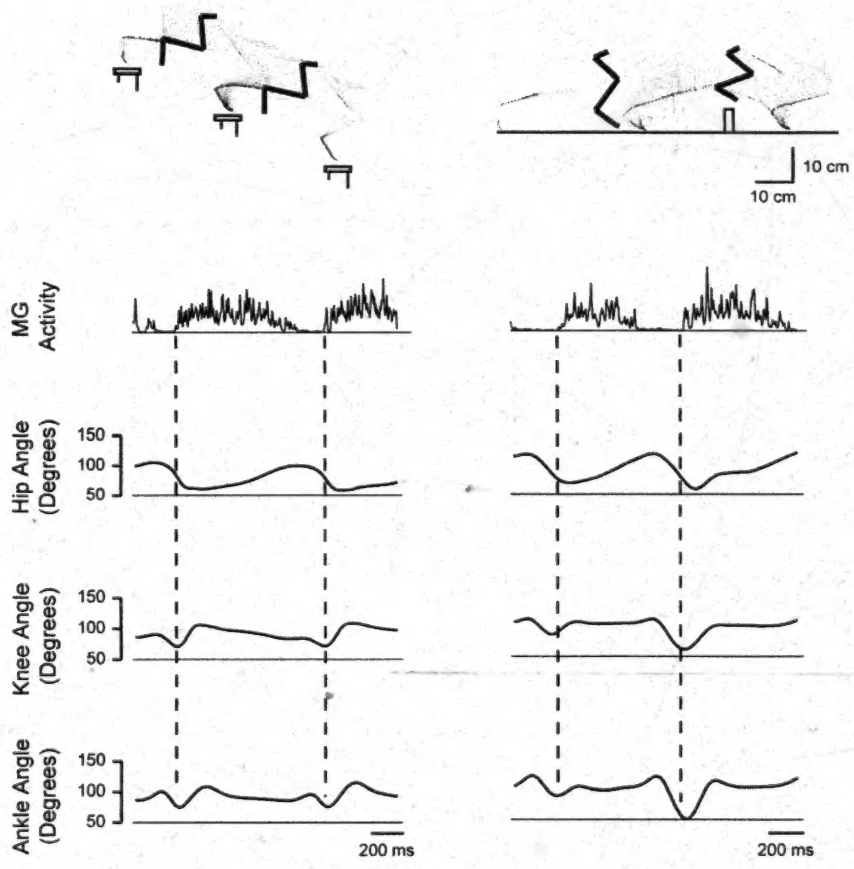


Figure 2.5

Example data from a 'down pegs' and a 'leading' trial. Top panels show stick figures of the hind leg at a rate of 60 Hz. Bold traces show the position of the leg at the time of onset of MG bursts. Lower panels show rectified and filtered MG EMGs and angles of the hip, knee and ankle. Dotted lines indicate the time of MG bursts. Note that the angle of the hip was relatively constant at the time of MG activation, despite the variation in the interval between the onset of MG bursts and ground contact (as seen by the position of the bold traces in the top panels relative to the position at ground contact). Also note the variation in the knee and ankle angles at the time of onset of MG activity.

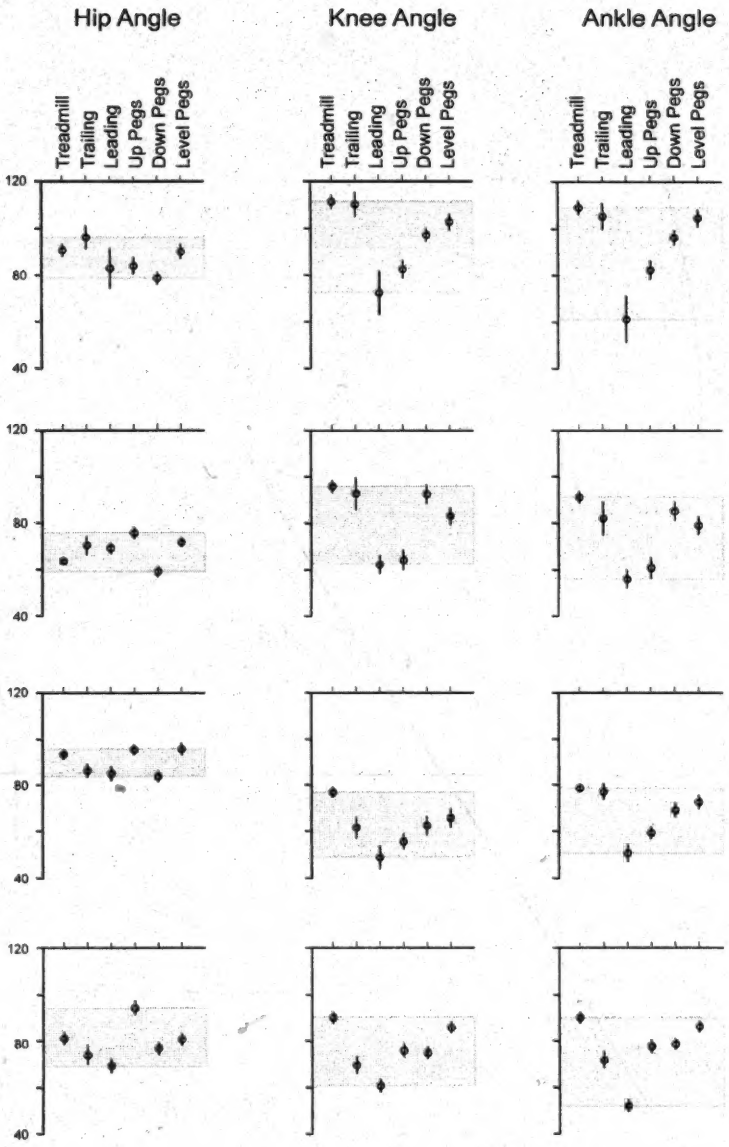


Figure 2.6

Hip angle is the least variable joint angle at the onset of extensor activity during the swing phase. The three main columns show the angles of the hip (left), knee (middle) and ankle (right) at the time of onset of MG activity for the four experimental animals (each row represents one animal). For each joint the data for the different tasks are shown in six adjacent sub-columns. Circles represent means and bars represent 95% confidence intervals for each task. Shaded area represents the range of the means of all six tasks. This range is smallest for the hip for all animals. See Figure 2.9 for the number of trials for each condition.

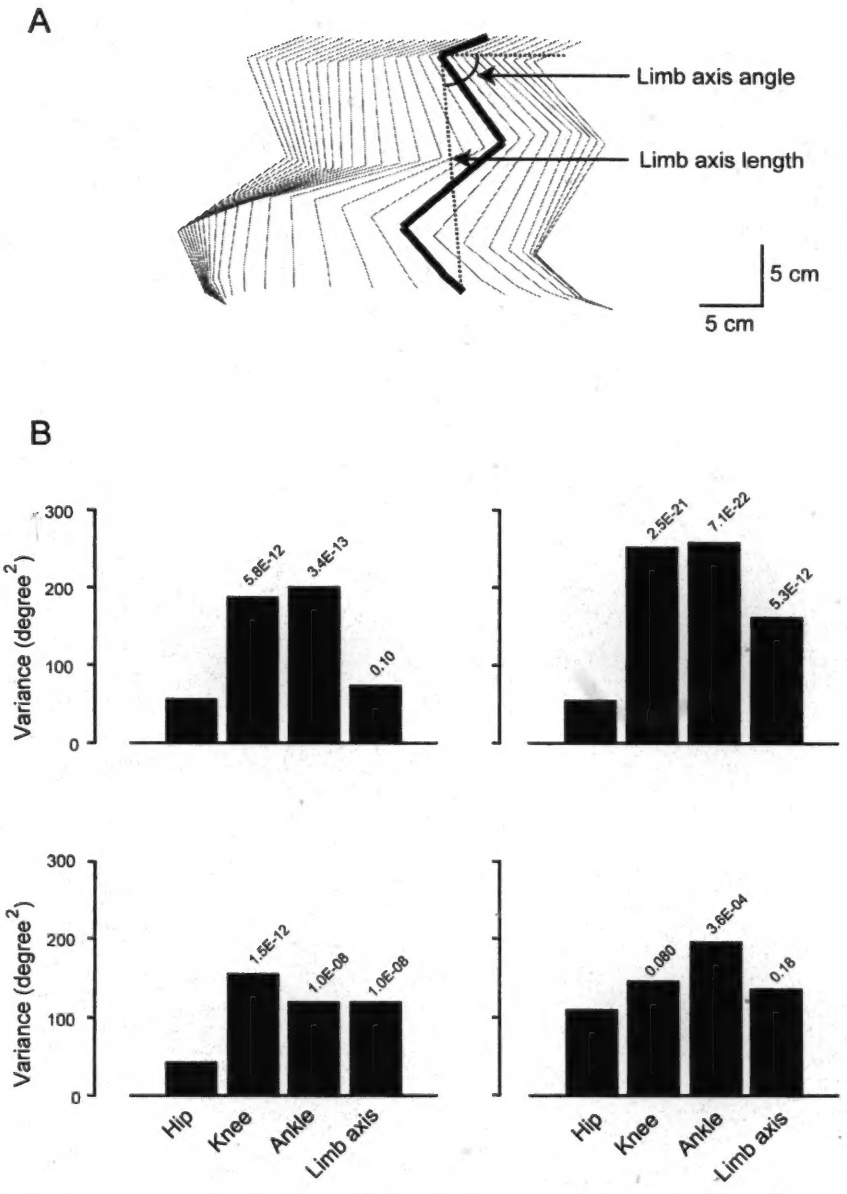


Figure 2.7

Hip angle at the time of the onset of extensor activity is less variable than other joint angles or limb axis angle. **A.** Stick figure defining limb axis angle length (see Figure 2.8 for data on limb axis length) is the distance from the hip to the toe. **B.** Variance of joint and limb axis angles at MG activation across all conditions. Each graph shows data from one animal. Number of trials for each animal is the sum of trials shown in Figure 2.9. F statistic above columns for knee, ankle and limb axis angle is result of two-tailed F-test testing for equality of variance between hip angle and that angle.

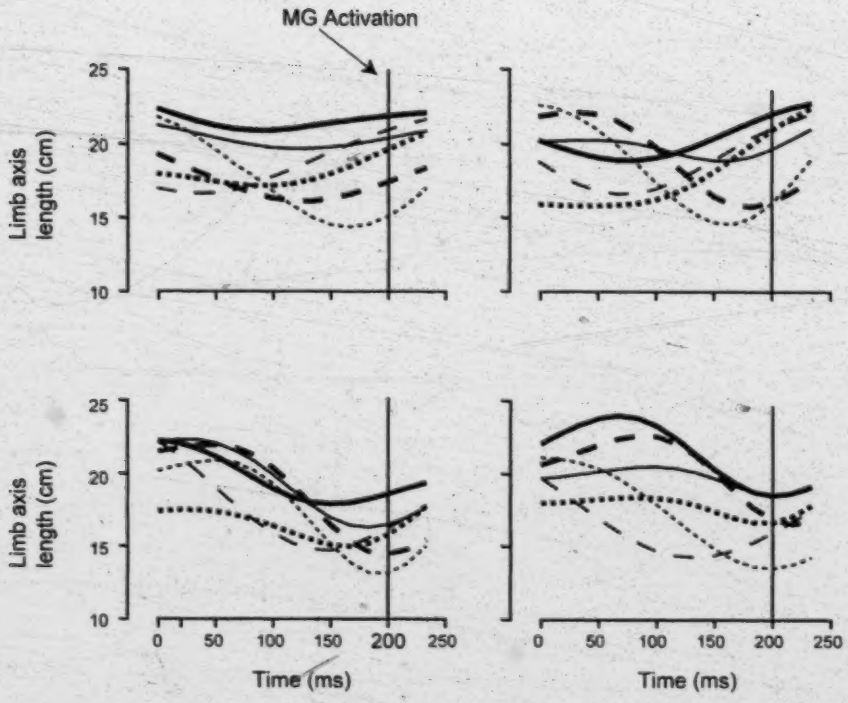


Figure 2.8

Limb axis length at the time of MG activation varies with locomotor task. Average limb axis length throughout one step for different tasks. Each trace is the average of between four and 15 trials, time-locked on MG activation (shown as vertical line at 200 ms.). The pattern of limb axis length through steps differs from task to task, as does the length at the time on MG activation. Thick solid – treadmill; thick dashed – up pegs; thick dotted – down pegs; thin solid – level pegs; thin dashed – trailing over object; thin dotted – leading over object.

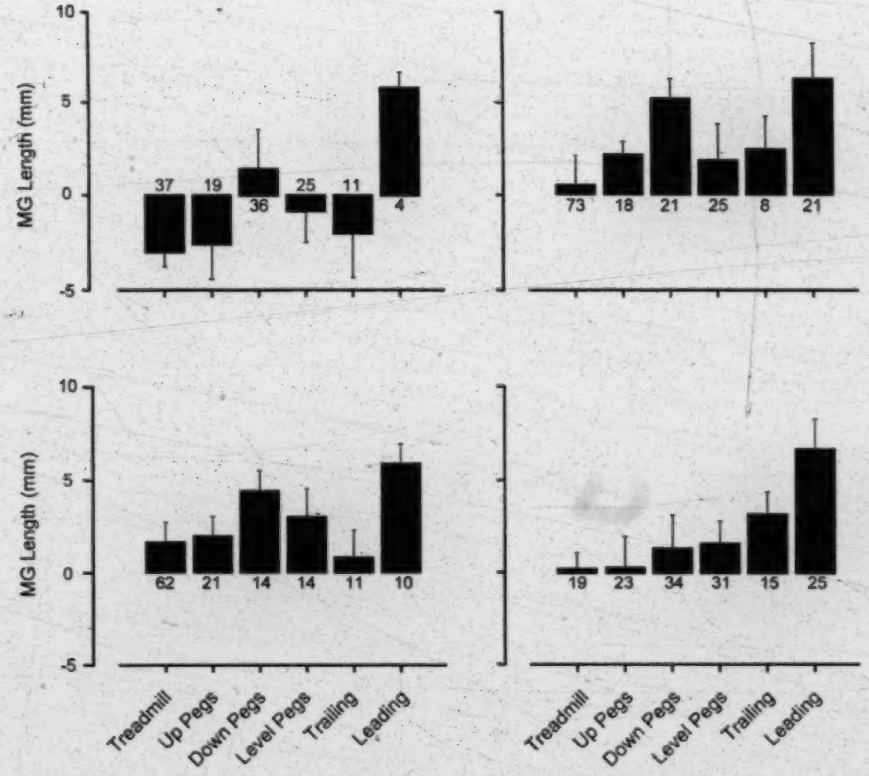


Figure 2.9

The length of the MG muscle at the time of MG activation varies from task to task. Muscle length was calculated relative length when knee and ankle angles are 90° (see Methods). Each set of graphs shows the average length of MG at the time it became active during different tasks for one animal. Error bars represent one standard deviation, and the numbers indicate the number of trials. Panels correspond to those in Figure 2.6 and 7, thus these numbers also indicate the number of trials for Figures 2.6 and 2.7.

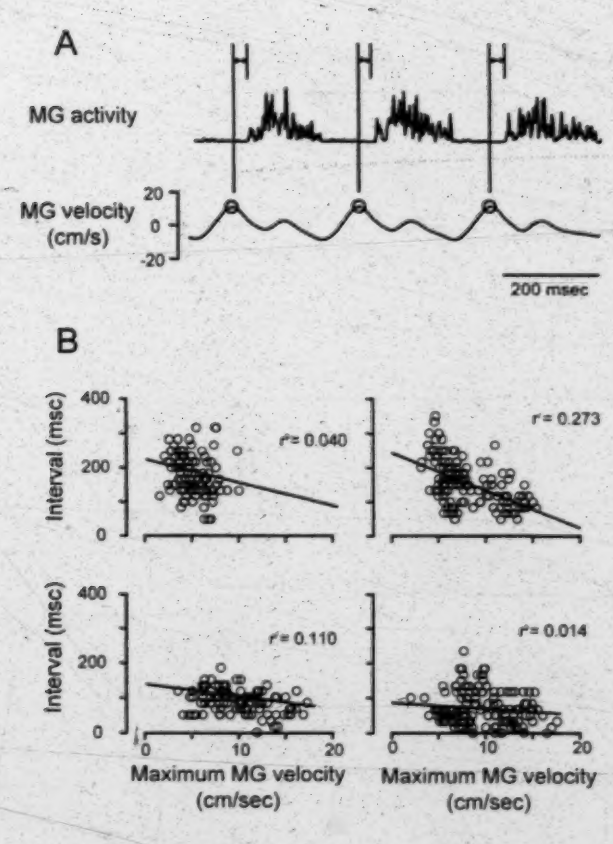


Figure 2.10

Maximum lengthening velocity of MG is not correlated to the time from maximum lengthening velocity to the time of MG activation. **A.** Three step cycles showing the measured parameters. Open circles show maximum lengthening velocity in MG during the first half for the swing phase, while the arrowheaded lines show interval from maximum velocity to MG activation. **B.** Relationships between maximum lengthening velocity in MG and interval from maximum velocity to MG activation for the four experimental animals. Data from all six tasks are combined in each plot. Results of linear regression are also shown. Note the maximum intervals are large, and the relationship between the two parameters is weak.

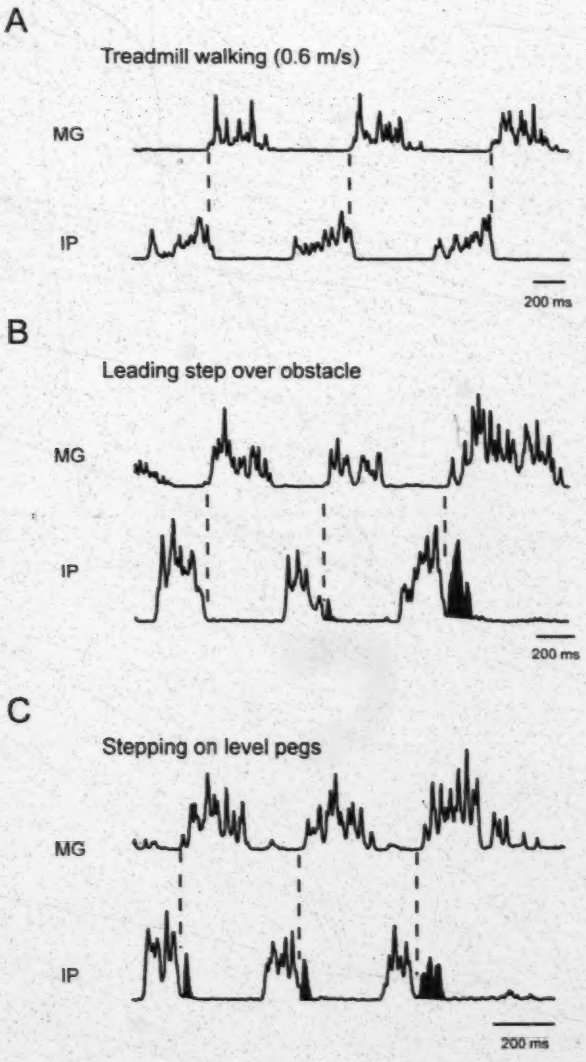


Figure 2.11

Activity of IP in intact animals can overlap the onset of activity in MG. Records show rectified and filtered EMGs from IP an MG during short sequences of walking during three tasks. **A.** Walking on treadmill. **B.** Leading over an object (third step is over object). **C.** Walking on pegs. Note that there was no overlap of the IP and MG bursts during treadmill walking, but clearly discrete bursts of activity in IP (shaded black) overlapped MG activity when the animal stepped over the object and when it walked on the pegs. These bursts began close to the time of the onset of MG activity (dotted lines).

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Chapter 3[3]

3.1 Introduction

One essential task of the locomotor system is to avoid obstacles that impede walking. Important insights into the use of visual information for this task have come from the study of gaze direction in humans stepping over objects (Patla and Vickers, 1997) and with prescribed foot placement (Patla and Vickers, 2003). More detailed information about the neural structures responsible for this important behaviour have come from studying walking in the cat, because it is possible to record simultaneously from supra-spinal regions and multiple muscles while the legs pass over a variety of obstacles (see Armstrong and Marple-Horvat, 1996 and Drew, Jiang and Widajewicz, 2002 for reviews). One common finding from research in both humans and cats is that visual information about obstacles is not always used for on-line guidance or adjustment of leg movements. For example, humans walking towards an obstacle can accurately avoid it if they see it five steps before reaching its location (Mohagheghi, Moraes and Patla, 2004) and cats walking through a cluttered walkway can avoid obstacles for 3-4 steps in the absence of vision (Wilkinson and Sherk, 2005). That online visual guidance is not always used is particularly obvious in the control of the hindlegs of the cat, which encounter an obstacle after the head and eyes have passed beyond its position (Drew et al., 1996).

The aim of the current study was to explore some aspects of the obstacle memory that guides the movements of the hindlegs of the cat over obstacles (because we are studying a previously undescribed aspect of a normal behavior, we use the simple term

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McVea DA and Pearson KG. Long-lasting memories of obstacles guide leg movements in the walking cat. *J.Neurosci.* 26: 4: 1175-1178, 2006.

obstacle memory throughout this study to refer to some persistent internal representation of an obstacle encountered during walking). Our first objective was to determine whether this obstacle memory could persist for longer than the time periods described in previous studies on obstacle avoidance during locomotion. To test this, we had cats walk partway over an obstacle and stop, with only the forelimbs having crossed the obstacle. The obstacle was subsequently dropped away while the animals were distracted, and we examined the kinematics of the hindlimb when the animal resumed walking. We next asked whether this obstacle memory can guide hindleg movements over multiple obstacles. This portion of the study allowed us to investigate the detail with which the memory of obstacles encountered during locomotion is encoded. To examine this issue, we observed cats while they walked partially over two obstacles before pausing to eat. One obstacle was immediately lowered, and the subsequent kinematics when the animal resumed walking allowed us to make inferences about the precision of the memory guiding the hindlegs.

The main findings of this study are that memories of obstacles encountered during locomotion are very persistent, lasting as long as the animal remained standing over the original location of the obstacle. Furthermore, these memories are encoded in a way which represents precise spatial information about the obstacle location.

3.2 Methods

In this study, we used two protocols to study the obstacle memory of walking cats. All procedures were done with two cats, and were approved by the Health Sciences Animal Welfare Committee at the University of Alberta.

3.2.1 Protocol 1: One obstacle

In this protocol, cats were trained to walk partway over an obstacle 3.2 cm wide (the height of the obstacle was varied between 3 and 7.2 centimeters). Animals were stopped from walking forward after their front legs had crossed the obstacle by presenting them with a food dish with sufficient food for them to eat for several minutes. As soon as the animal began eating in this position, the block was lowered so that the top became flush with the walkway surface. Between five seconds to ten minutes after the animals straddled the obstacle, the animals were encouraged to walk forward so the hind legs crossed where the obstacle had been.

3.2.2 Protocol 2: Two obstacles

In this protocol, a second obstacle, which measured 3.5 cm wide by 6.5 cm high, was placed in front of the same movable obstacle described above, and was not lowered. The distance between the first (fixed) and second (lowered) obstacles varied from trial to trial, but the first obstacle was consistently placed so that a normal, unaltered step over it would intersect the position of the second obstacle. Cats were walked forward and presented with food so that both front feet crossed both obstacles, and one or both hind feet remained behind both obstacles. As in the one obstacle protocol, the movable

obstacle was dropped away and the animals were encouraged to walk forward after a delay period, which varied from trial to trial.

3.2.3 Training

Although cats were familiarized with the laboratory and the equipment described above, they were not trained to stand during the delay period. This allowed us to study a memory that was a natural part of locomotion, but had the disadvantage that we could not continue trials beyond the duration for which animals would naturally stand. Many trials were unsuccessful because animals spontaneously sat down or walked backwards.

3.2.4 Data collection and analysis

Reflective markers (diameter about 0.5 cm) were placed over the toe of the left leg, and the inner toe of the right paw. These markers were used to determine the kinematics of foot movements using the Peak Motus 8.2 motion analysis system (ViconPeak, Colarado). Data were collected over a period of three to four weeks for both animals. Trials were conducted every one to two days, for between 30 and 45 minutes. The entire testing session was recorded to video tape, and was later digitized off-line using the Peak Motus system. Trials in which the cat sat down, moved backwards, or looked away from its food dish for an extended period of time, were discarded.

3.3 Results

3.3.1 Memories are persistent

When cats step over an obstacle while walking, they produce a consistent pattern of hyperflexion of the fore- and hind-legs (Drew, Jiang and Widajewicz, 2002). We first tested whether the increased flexion in the hind legs would be present if the animal had the opportunity to see the obstacle, but was paused for a delay period after the forelegs, but not the hindlegs, had stepped over the obstacle. In other words, we asked if the obstacle memory that guides the hindlegs of cats over obstacles during normal walking could persist during long delays. Figure 3.1 shows the maximum step height following a delay period over an obstacle between 5.6 cm and 7.2 cm tall (there was no influence of obstacle height on maximum step height for this narrow range). These data show that cats will step significantly higher than normal even if paused for over three minutes in one animal, and up to ten minutes in another, before stepping over the obstacle. The trials in Figure 3.1 represent all of the trials in which the animals remained standing and did not sit or turn around.

3.3.2 Memories reflect detail of the object and its position

We next examined the precision of the obstacle memory guiding the increased flexion in the step over the location of the obstacle. First, using obstacles of different heights, we found that step height after a delay period depended on the height of the obstacle, shown in Figure 3.2A. Secondly, Figure 3.2B shows that the trajectory of the cat's toe towards the obstacle varied depending on the distance between the toe and the

obstacle at the time the step was initiated. Third, in trials involving two blocks (one of which was dropped away at the beginning of the delay), the paw generally landed between the two obstacles and subsequently passed above where the second obstacle had been, or passed above both obstacles in a single step (Figure 3.3A). Occasionally, we observed a striking example of the detail with which foot movements were guided. Figure 3.3B shows two examples. In the upper example, the foot was stopped and redirected in mid-swing to avoid crossing the previous position of the obstacle. This step followed a delay period of four seconds. In the lower panel, the foot was lifted vertically until it was above the height of the remembered obstacle before being moved forward and over the previous position of the obstacle. This step followed a delay period of ten seconds

3.4 Discussion

Although walking animals rely on visual input to avoid obstacles, vision is not always used for online guidance of leg movements. Both humans and cats can step accurately over obstacles they have not seen for a few seconds during normal walking (Mohagheghi, Moraes and Patla, 2004, Patla and Vickers, 1997, Wilkinson and Sherk, 2005). In this study, we examined the duration and precision of one form of this memory, which, in cats, guides the hindlegs over an obstacle once the forelegs and the head have passed over it. Our strategy was to examine the leg movements of cats that had been paused for a delay period after the forelegs, but not the hindlegs, had stepped over an obstacle.

Our initial question was whether the memory of an obstacle encountered during walking would persist during an abnormal delay period, to allow subsequent steps to

avoid the obstacle. The hindlegs of normal walking cats encounter obstacles up to 800 ms after the forelegs during walking (Drew et al., 1996). As Figure 3.1 shows, increased flexion was evident during all delays we examined. In most cases, this was between ten and two hundred seconds, as this was the length of time animals usually remained standing. In a few cases, however, the delay exceeded two hundred seconds, lasting six hundred seconds in one case. Large flexions were evident even following these exceptional delays. We were not able to determine the upper limit of the duration of this memory. Our attempts to test delays longer than a few minutes were usually prevented by the difficulty of keeping a cat standing and eating in one position for long periods of time. Nevertheless, we anticipate the memory of the obstacles may last considerably longer than ten minutes, because the one trial we obtained with a ten minute delay showed no decline in magnitude compared to the trials at shorter delays. Furthermore, we found no sign of a progressive decline over a period of three minutes in either animal, indicating this form of memory is very stable. We can be confident the altered step was driven by memory, and not by vision or somatosensory feedback, because the obstacle was absent during the delay period.

These long delays are at least an order of magnitude longer than those typically used in working memory tasks (Pasternak and Greenlee, 2005). For example, a recent pointing task to a remembered target noted decreases in accuracy after a 10 second delay (Lemay and Proteau, 2001), while a grasping study examined the effects of a five second delay on accuracy (Hu and Goodale, 2000). A recent study involving cats' working memory of hidden objects found accuracy declined rapidly between delays of 0 and 30 seconds (Fiset and Dore, 2005). The memory we describe also lasts much longer than

those described in other locomotion tasks which rely on memory. For example, cats can step accurately through a cluttered walkway for between 2 and 3 seconds when relying on memory (Wilkinson and Sherk, 2005).

One possible explanation for the much longer-lasting memory of this study is that, during these long delays, animals held a simple memory that their next step would have to be high. This would be simpler and presumably more robust than a memory which carried detail about the obstacle and its position, and which guided the animals' movements once the step began. Four observations suggest the former is not the case. First, the height of the high step following the delay period varied depending on the height of the obstacle (Figure 3.2A). Second, Figure 3.2B shows that the trajectory of the cats' toe towards the obstacle varied depending on the distance between the toe and the obstacle at the time the step was initiated. This suggests that, during the delay period, the cats were aware of the position of the obstacle relative to their feet. The third observation that supports the contention that the high steps were guided by a detailed spatial memory of the obstacle came from trials involving two obstacles. In these trials, the large majority of steps took one of two trajectories – either landing between the two obstacles or passing above both. In the former case, the cat would have to make two altered steps to avoid the obstacles – first dropping the foot sharply after crossing above the first obstacle, and then stepping higher than normal on the subsequent step to avoid the position of the second obstacle (which had been lowered). Only rarely did the toe pass through the position where the obstacle had been before it dropped away (Figure 3.3). Because the cats we examined stepped *either* over both obstacles, *or* between them in such as way that they did not pass through where the obstacle had been, a simple memory of the obstacle that

did not include information about its size and position would not be sufficient. Finally, the obstacle memory present during the delay period could be used to perform online corrections of stepping movements, as shown in Figure 3.3B. Note that these corrections were matched closely to the previous location of the obstacle. These four lines of evidence reflect a memory involving a precise representation of the obstacle relative to the body.

Given that the memory examined in this study includes information about the size and position of the obstacle relative to the body, it is worth considering why this memory lasts for significantly longer than either spatial memories examined in the context of reaching or saccades, or other forms of memories of obstacles during locomotion. One potentially relevant aspect of the memory we describe here is that the animal has both visual input about the size and shape of the obstacle, and proprioceptive input related to the stepping of the forelegs. It may be that the action of the forelegs stepping over the obstacle provides essential information for establishing this memory. This combination of visual and proprioceptive input may be an important difference between this and other shorter-lived memories which guide movements during locomotion. Another relevant fact is that the memory task we used involves a natural, familiar task (stepping over an obstacle) interrupted mid-way. Furthermore, this memory task did not require the animals' attention. Unlike working memory tasks, most of which require the animals' attention (for example, Fiset and Dore, 2005; for review, Pasternak and Greenlee, 2005 and Goodale, Westwood and Milner, 2004), the cats we studied were focused on their food as they ate, and not on the task. Importantly, they were not trained to stand or avoid the obstacle. One intriguing possibility, then, is that we are describing an essential feature

of locomotion in cats, which is short-lived during normal walking but can be extended for long periods of time. Cortical recordings from cats walking over obstacles provide some clues as to what areas of the nervous system might be involved in this behaviour. Widajewicz *et al.* (1994) described cells in the motor cortex which were active for the interval after the forelimbs but before the hindlimbs had passed over an obstacle during normal, uninterrupted walking. It remains to be seen whether similar activity is evident in the motor cortex and other brain regions throughout the delayed stepping we describe in this study. If cortical regions are found in which neuronal activity is related to obstacle memory, then memory-guided stepping may provide an excellent paradigm for investigating a simple and robust form of working memory.

3.6 Figures

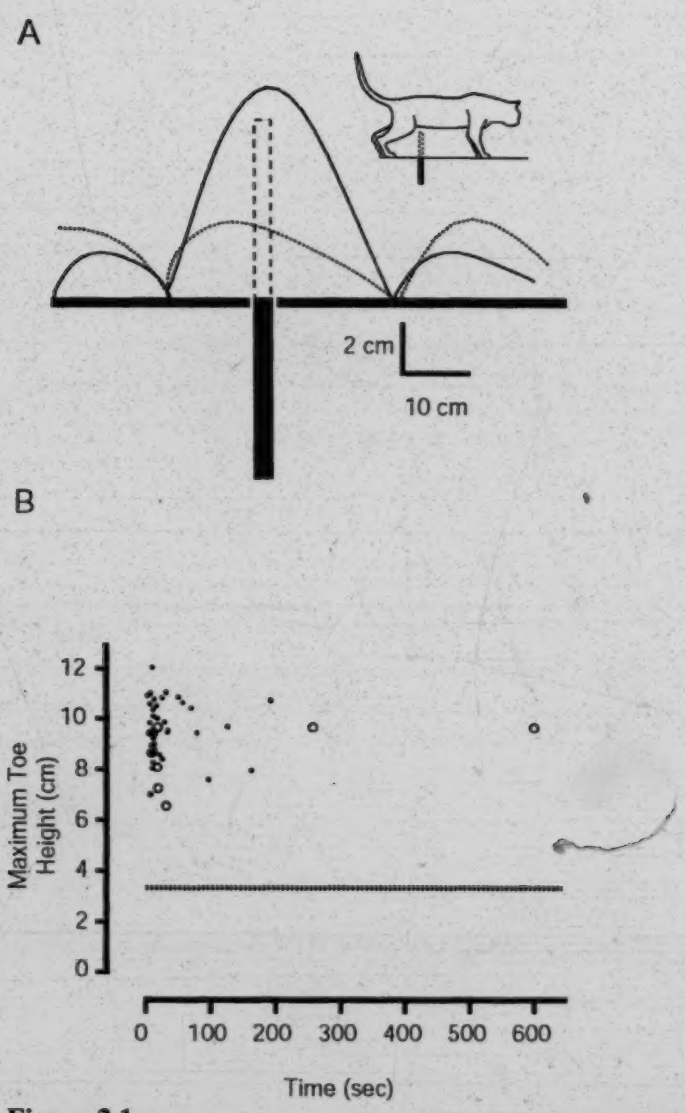

Figure 3.1

A) Toe trajectory during a step over a remembered obstacle (solid line). Delay period was twenty seconds. Diagram in upper right corner shows the experimental set up. Cats stepped over an obstacle with the forelegs. The obstacle was lowered while the animal stood still eating. Dotted line shows trajectory of a normal step without the obstacle for comparison. Dashed outline shows original position of obstacle. B) Maximum toe height during the step after a delay over a remembered obstacle (height varied between 5.6 and 7.2 cm) for a variety of delays. Open circles and dots represent different animals. Dashed bar is the mean toe height (n = 8) during control steps for the cat represented by the dots. Mean of control steps (n = 9) for cat represented by open circles was 3.1 cm (not shown).

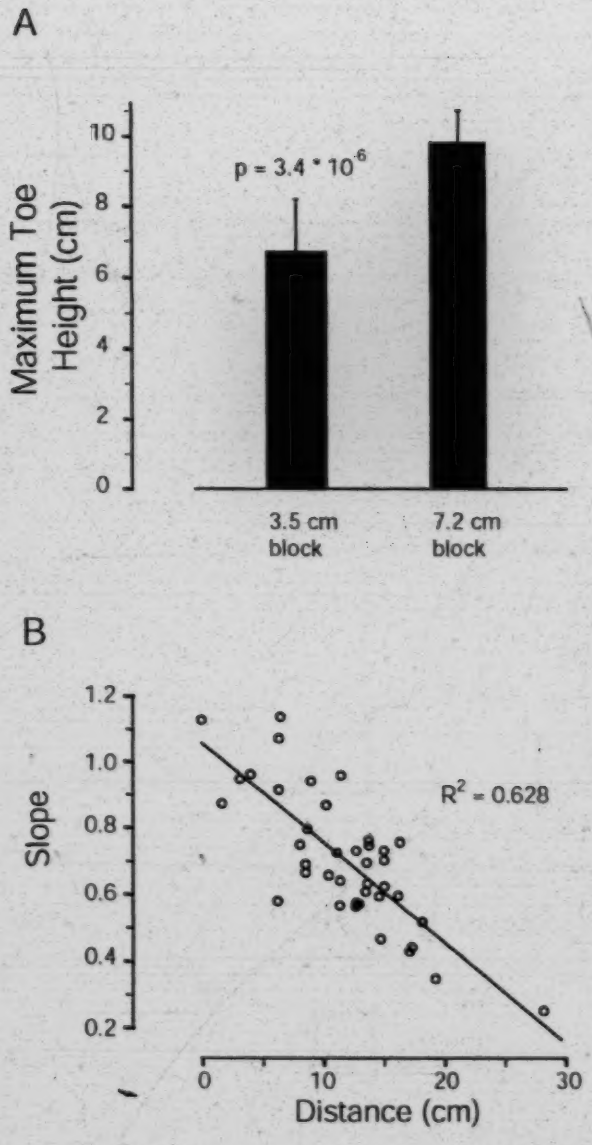

Figure 3.2

Steps of the hindlimbs after a delay over a remembered obstacle depend on the position of the toe and the size of the block. A) Maximum toe height during the step after a delay over a remembered obstacle changes for different obstacle heights. B) Slope from the toe position at the start of the step to the toe position at the maximum height varies inversely with distance from the remembered obstacle at the time of step initiation.

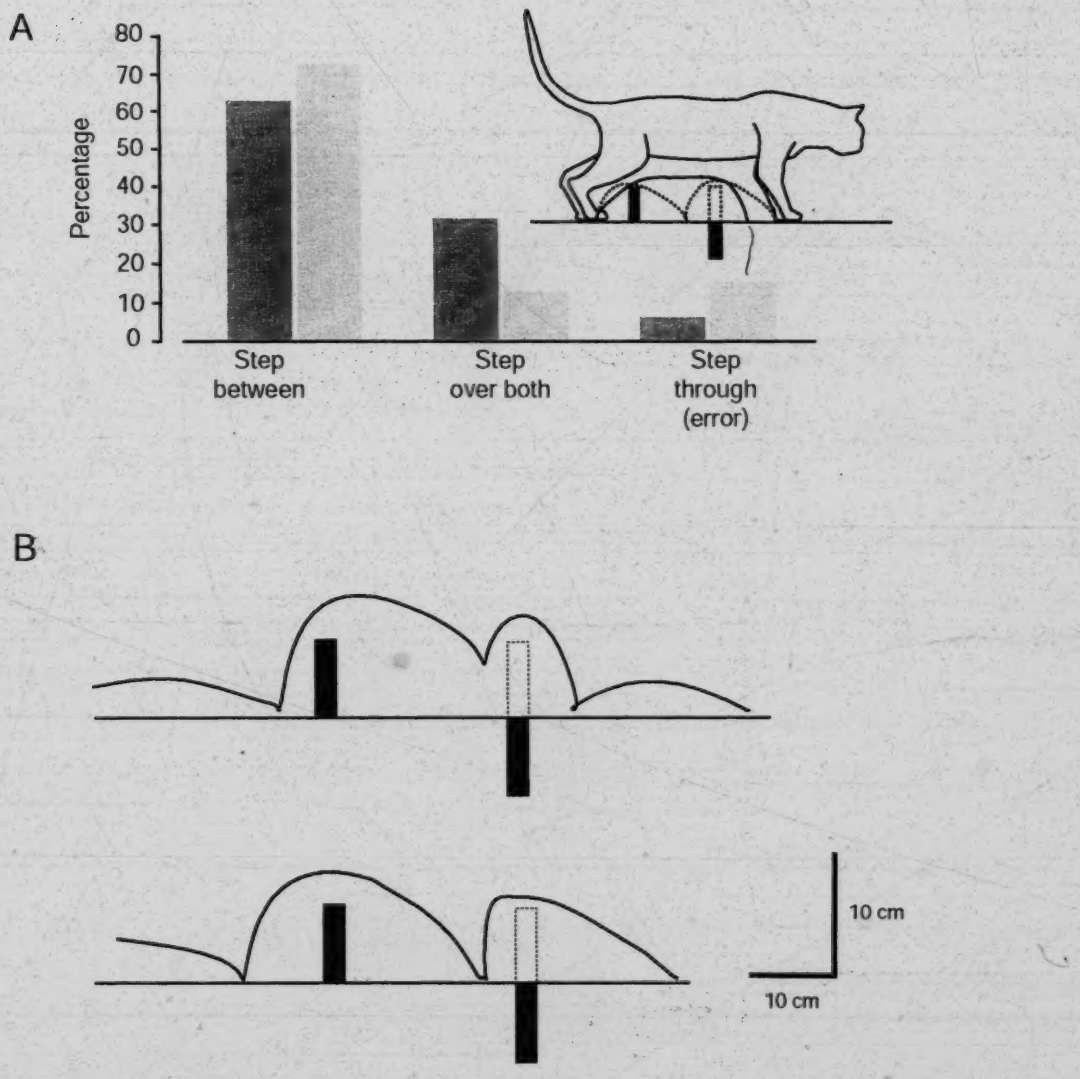

Figure 3.3

During stepping after a delay over two obstacles, cats demonstrate memory of obstacle location. A) Steps following a delay over two remembered obstacles show memory of both obstacles. Inset shows protocol. The animal was stopped and fed after the forelegs had crossed both obstacles, and the hindlegs had crossed one or neither obstacle. Immediately after, the right obstacle was lowered and the animal's forward progression was delayed by feeding. During the step following the delay, the animal could *Step between* (dashed), *Step over both* (solid) or step through the former position of the lowered obstacle (not shown). Different shades represent two cats. Dark gray = 78 steps, light gray = 40 steps. B) Toe trajectories over two blocks showing precise memory of lowered block location. Upper panel: a leading step in which the animal reversed direction in mid-swing, very close to the original position of the lowered block. Lower panel: a trailing step in which the animal lifted the toe nearly vertically until it was just above the former position of the obstacle, before moving it forward.

3.7 References

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Chapter 4[4]

4.1 Introduction

As animals move through the environment, they encounter uneven terrain, obstacles, and other impediments to their progress. The unique requirements of each of these challenges highlight the need for flexibility in the neuromuscular control of locomotion. Two broad categories of adaptations are used to this end (Pearson 2000). The first is a step-to-step adjustment of motor activity to accommodate the immediate features of the environment. For example, visual signals are used for rapidly adapting stepping to avoid obstacles (Drew et al. 2002, McVea and Pearson 2006, Patla and Vickers 2003), while proprioceptive signals are used to regulate activity in extensor muscles depending on the load carried by the leg (Donelan and Pearson 2004a, Donelan and Pearson 2004b). The second category of adaptation employed by walking animals adjusts the pattern of motor activity during walking to accommodate a persistent change in the environment. Many forms of such adaptation have been described. For example, humans adjust the duration of the swing and stance phases while walking on split-belt treadmills (Prokop et al. 1995), rotating discs (Weber et al. 1998), and treadmills with rapidly changing speeds (Rand et al. 1998). In this study we report a form of adaptation in this second category, namely a persistent modification of the swing movement of the hind leg of cats to avoid an obstacle that repeatedly perturbed the swing movement.

This adaptive response was discovered in the course of an investigation on sensory regulation of the swing-to-stance transition (McVea et al. 2005). In that

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[4] A version of this chapter has been submitted for publication:
McVea DA, Pearson KG. Long-lasting and context-dependent adaptive modification of stepping in the cat, *Journal of Neurophysiology*

investigation we attempted to enhance swing movements of a hind leg by mechanically stimulation the dorsum of the paw during the swing phase. We unexpectedly observed that repeated mechanical stimulation of the paw led to an increase in knee flexion and step height (we will refer to this behavior throughout this paper as *persistent hyperflexion*) even in the absence of further stimuli (not reported in McVea et al. 2005). The hyperflexion of the leg in response to mechanical stimulation of the paw is often referred to as the "stumbling-corrective response", and previous investigations in cats have documented the neural pathways and changes in leg kinematics and muscle activity underlying this response (Forssberg 1979, Quevedo et al. 2005, Wand et al. 1980). None of these studies, however, have examined adaptive modifications following multiple steps obstructed during swing in normal walking cats, although brief after-effects (lasting for one to ten steps) following repeated stumbling-corrective responses have been reported in infants (Pang et al. 2003), decerebrate ferrets (Lou and Bloedel 1988a) and spinalized cats (Edgerton et al. 2004). In contrast, the after-effects we describe in this study lasted for much longer periods, over 24 hours in some cases.

The discovery of the persistent conditioned hyperflexion during treadmill walking raised the question of whether this adaptive behavior is context-dependent. Context-dependent modification of locomotor behavior has been previously been reported in cats walking on inclined walkways (Carlson-Kuhta et al. 1998, Smith et al. 1998a) and humans walking on a compliant and slippery surface (Maclellan and Patla 2006, (Marigold and Patla 2002). To investigate this issue we conditioned the hyperflexion while animals walked on a treadmill and then examined leg movements when animals walked in a different environmental context. In addition, we tested whether or not the

persistent hyperflexion could be evoked in decerebrate walking animals and following denervation of the main flexor muscles of the knee, posterior biceps and semitendinosus. Our main findings were that the conditioned hyperflexion is context-dependent, and that it cannot be produced in either decerebrate walking animals or after partial denervation of the main knee flexor muscles.

A brief report of some of the findings in this study has been published in abstract form (McVea et al. 2004).

4.2 Materials and methods

The Health Sciences Animal Welfare Committee at the University of Alberta reviewed and approved all of the procedures involved in this study.

4.2.1 Surgical procedures

The main objective of this study was to investigate the long-term after-effects following a series of stumbling-corrective responses during walking in the cat. To do this, six adult cats were trained to walk on a custom-built treadmill at speeds ranging from 0.3 m/s to 1.0 m/s. Once cats were walking confidently and reliably, they were anesthetized (Isoflurane) and bipolar recording electrodes (Cooner wire AS632) were implanted unilaterally in the hind leg under aseptic conditions. The muscles implanted varied from animal to animal, but generally were the ankle extensors soleus (SOL), lateral gastrocnemius (LG), medial gastrocnemius (MG), the knee extensor vastus lateralis (VL), and the hip flexor iliopsoas (IP), and semitendinosus (ST, knee flexor/hip extensor) muscle. Sartorius (Sart), a hip and knee flexor, was implanted in two animals. For this project, only data from the IP, ST, and Sart muscles were analyzed. Data recorded from other muscles were used in unrelated projects. A multi-pin socket attached to the animal's skull using dental acrylic allowed access to the electrodes.

In two animals (cats 1 and 3) a second surgical procedure was performed 35 and 43 days later, respectively. After anesthetizing with Isoflurane, the nerves innervating ST, posterior biceps (PB) and anterior biceps (AB) of the right hind limb were cut just distal of their divergence from the sciatic nerve to denervate the main flexor muscles of the

knee. The nerve innervating AB (hip extensor) was included because it includes motor axons supplying PB and ST (English and Weeks 1987).

4.2.2 Stumbling-corrective responses

A key element of this study was initiating repeated stumbling-corrective responses in walking cats. To do this, we used a hand-held wooden rod, approximately 1 meter long, with a 4 cm long screw fixed perpendicularly in one end to form a hook. The screw was wrapped in gauze and medical tape to pad it. This hook was positioned manually to contact the dorsum of the foot early in the swing cycle while cats were walking on a treadmill (Figure 4.1). In this paper, we will refer to each stimulus as a *perturbation*. Animals quickly became familiar with this process and would walk comfortably during repeated perturbations. We generally did not position the hook at a consistent level, because steps became progressively higher through a sequence of perturbations so it was necessary to raise the hook to continue to elicit flexion responses. As much as possible, we restricted contact of the hook to the dorsum of the paw.

4.2.3 Investigation of context specificity

In five animals, we observed walking in contexts different from the treadmill to ascertain whether an increase in step height resulted from increased flexion in all walking conditions. In these experiments, the steps of cats were repeatedly perturbed while walking in a treadmill until they were exhibiting persistent hyperflexion. We then compared the expression of the persistent hyperflexion in the treadmill and in a different context (either a level walkway or a series of offset pegs). Usually, this comparison was

made immediately. In other cases, when the persistent hyperflexion was still evident a number of days after the perturbed steps, the comparison was made days later.

In three animals, we delivered a sequence of perturbations at a specific speed (0.5 or 0.6m/s) and then observed walking at different speeds, to determine whether the persistent hyperflexion was specific to one speed.

4.2.4 Stumbling-corrective responses in decerebrate walking cats

The general procedure for the decerebrate walking preparation has been described extensively in previous publications from our laboratory (Donelan and Pearson 2004b, Hiebert and Pearson 1999). Two animals were anesthetized with Isoflurane and a tracheal cannula inserted for continued administration of the anesthetic. A cannula, inserted into one of the carotid arteries, was used to monitor blood pressure. Drugs and fluids were delivered via a second cannula inserted into one jugular vein. Bipolar recording electrodes (Cooner wire AS632) were then sewn into the lateral gastrocnemius (LG), medial gastrocnemius (MG), the hip flexor iliopsoas (IP), and semitendinosus (ST, knee flexor/hip extensor) muscles of one hind leg. Data from recordings from LG and MG were used for unrelated projects. A multi-terminal connector positioned above the animal's back allowed recordings of muscle activity throughout the experiment. To support the animal's hindquarters, a thick wire was threaded through holes drilled in both iliac crests. This wire was later clamped to an external frame to support the hips while the animal was walking on a treadmill. Reflective markers were placed above the iliac crest, the hip joint, knee and ankle joints, and on the paw and toe of the left leg. These markers

were used to determine the kinematics of leg movements using the Peak Motus 8.5 motion analysis system (VicomPeak).

Following these surgical procedures, the animal was transferred to a frame mounted above a treadmill. The wire through the iliac crests was clamped to an external frame, and the head was placed in a sterotaxic holder. The brainstem was subsequently transected rostral to the superior colliculus and mammillary bodies, and the animal was removed from the anesthetic. Both animals began to walk spontaneously about 30 minutes later.

During regular walking sequences, a hook similar to that described above for use in intact cats, was used to perturb the animal's leg during the swing phase of walking.

4.2.5 Investigation of maintenance of persistent hyperflexion following decerebration

In one cat, we repeatedly perturbed stepping to elicit persistent hyperflexion (between 100-120 perturbations). We then performed a decerebration as described above, to determine whether any aspects of the persistent hyperflexion would be evident following decerebration. The decerebration was complete within four hours of the perturbed steps.

4.2.6 Data acquisition and analysis

Data were collected daily from intact animals for 10-14 days, and over the course of 1-3 hours from decerebrate animals. Both video and EMG signals were recorded through the entire course of each experiment; video was recorded using a Peak Motus system, and EMG was recorded with an 8 channel Vetter 4000A PCM recorder (band

pass filtered from 30 to 30,000 Hz). The Peak Motus system placed a signal periodically on both the video and the EMG recordings, which was used for off-line synchronization. The Peak Motus system was also used to track reflective markers (0.5 cm diameter) placed over iliac crest, the hip, the ankle, the paw and on the fifth digit of the toe. The position of the knee was calculated using triangulation from the hip and ankle locations and measurement of thigh and shank lengths. These data were used to calculate relevant kinematic data, such as joint position and angle and toe height above the supporting surface.

Custom programs written in Matlab (The MathWorks) were used to analyze the synchronized EMG and kinematic data. Kinematic data were first low-pass filtered (fourth-order Butterworth, zero-lag) and then analyzed to determine maximum step height, angle flexions, and other parameters of interest. EMG data were rectified, filtered (first-order Butterworth, zero-lag) and analyzed.

4.2.7 Statistical tests

Comparison of multiple means was done using the Kruskal-Wallis test, a non-parametric alternative to the ANOVA test. Comparison of two means was done using Student's t-test, with allowances for unequal variances.

4.3 Results

This study describes a long-lasting adaptation of the swing phase in walking cats. This adaptation was produced by repetitively evoking stumbling-corrective responses in one hind leg using a handheld device while animals walked on a treadmill (Figure 4.1). The main observation was that the flexion of the knee progressively increased during the perturbation sequence and that increase in knee flexion was long-lasting and context-dependent (throughout this paper we will refer to this behaviour as *persistent hyperflexion).* An easily quantified measure of this hyperflexion is the maximum height of the toe during the swing phase of walking, and we use this as a measure throughout this study. Of six cats studied, five showed a long-lasting increase in step height following such a sequence of perturbed steps. We noted two relevant differences between the cat that exhibited only temporary increases in step height and the remaining cats. The first was that this cat was much more timid and nervous in our laboratory, and the second was that this cat walked with a very crouched gait. For the remainder of the analysis and discussion, we will refer only to the five cats which did exhibit persistent hyperflexion.

4.3.1 Stumbling-corrective responses elicit long-lasting increases in step height

During undisturbed walking, cats show a regular pattern of flexion and extension in the joints of the legs. Normally, after contact with an object during walking, the basic pattern of leg movements is only briefly modified, a response described as the stumbling-corrective response (Forssberg 1979, Wand et al. 1980) as shown in Figure 4.1B. In work related to an earlier study in our laboratory (McVea et al. 2005), we made the observation that after sequences of repeated stumbling-corrective reactions, much longer-lasting

modifications resulted. Upon further investigation, we observed that the maximum step height increased markedly following a series of stumbling-corrective responses (henceforth referred to as *perturbations* or *perturbed steps* [defined in methods]), as shown in Figure 4.1C. An analysis of the results from four cats is presented in Figure 4.2. These data show that walking cats adapted swing movements rapidly following a small number of perturbed steps. For example, all cats increased their step height by at least one-third following only twenty perturbed steps. Larger numbers of perturbed steps produced a further increase in step height, and this increase in step height persisted for at least 24 hours in two animals (cats 2 and 3), and at least 20 minutes in the remaining animals when returned to the treadmill without further perturbations. Figure 4.3 shows that this increase in step height resulted from an increase in flexion at the knee following perturbed steps. Changes in flexion at the hip and ankle were not significant except in one animal in which ankle flexion increased. Figure 4.4 shows that another feature of the adaptation to repeated perturbations was an enhancement of the stumbling-corrective response. Even after the persistent hyperflexion had waned (Figure 4.4A), a perturbation during swing led to a series of higher steps, which was not evident in the control case (although the first step in the lower panel of figure 4.4A appears higher than control, this is only because the cat was taking long steps while moving forward through the treadmill. Figure 4.2 shows this cat did not have persistent hyperflexion lasting for over 24 hours). In one interesting case, a perturbation of the contralateral leg (which never developed persistent hyperflexion) led to a sudden increase in step height in the ipsilateral leg (Figure 4.4C). This was not observed in other animals.

EMG recordings from hip and knee flexor muscles were consistent with these observations on movements at the knee and hip; activity in ST, an important knee flexor, increased concurrently with step height (Figure 4.5A,B) while activity in IP, a hip flexor, did not (Figure 4.5A).

4.3.2 Expression of persistent hyperflexion is context-dependent

We next examined whether the persistent hyperflexion was expressed in all environments, or restricted to the environment in which the perturbations were encountered. We tested this by first perturbing the steps of cats as they walked on the treadmill until the persistent hyperflexion was evident (between 80-120 perturbed steps). We then compared the maximum step height during unperturbed steps in the original treadmill and in a new environment (either a series of offset pegs or a level walkway). In some cases, this comparison was done within a few minutes after the development of persistent hyperflexion, while in other cases, it was done some days later. Figure 4.6A illustrates the basic phenomenon, showing high steps following perturbations on the treadmill, much lower steps on a walkway, and a return of high stepping following return to the treadmill. Figure 4.6B presents averaged maximum step height from two more cats, showing a significant difference between the maximum steps height in the different contexts. Note in the data from cat 2 that increased step height on the treadmill persisted for at least three days following perturbed steps. Four of the five cats that displayed a persistent hyperflexion on the treadmill showed a context-dependent expression of the increase in step height. In the fifth, the step height after returning to the treadmill was

higher than the step height on the walkway, but the difference was not statistically significant.

Following this clear evidence that the expression of the persistent hyperflexion was restricted to certain contexts, we occasionally observed that particular contextual information was sufficient for initiating this expression. Figure 4.7A shows evidence that visual and auditory cues related to the animal's environment are one source of contextual information. Data in the upper panel show that step height increased markedly when the rod used for perturbing steps entered the treadmill. This was likely due to some combination of the animal seeing the rod being positioned close to its body and the sound of the rod being positioned in the treadmill. Although this phenomenon was not thoroughly examined, similar but smaller effects were seen in a second cat (not shown). In the lower panel, data from a sequence of steps in which cat 1 increased step height over the course of four steps after the treadmill was engaged are shown. In this case, sensory cues related to the sound of the treadmill engaging, or the movement of the treadmill under the animal, were likely to be source of the contextual information. There was no stimulus applied to the paw in either the upper or lower panel of Figure 4.7. We also obtained evidence in one cat that the speed of walking was a cue that triggered expression of the increased maximum step height. Data from this cat is shown in Figure 4.7B. Note that the step height at 0.5 m/s, which is the speed at which the animal initially experienced the perturbed steps, was the highest over the range of speeds tested. In contrast, the step height of the contralateral toe changed very little across these different speeds, supporting our conclusion that the peak in step height at 0.5 m/s is a context-dependent adaptation to the prior perturbed steps. We believe the change in step height

was related directly to the speed of the treadmill, and not to other cues such as verbal announcements of treadmill speed, because of the partial increase at speeds close to 0.5 m/s.

4.3.3 Main knee flexor muscles are essential for long-term retention of persistent hyperflexion

As Figure 4.3 shows, knee flexion increased significantly in cats following a sequence of perturbed steps, and this increase was due at least in part to an increase in ST activity (Figure 4.5). As part of a concurrent project in our laboratory concerning the recovery of flexion movements following the loss of knee flexor muscles (Tachibana et al. 2006) we were able to examine the ability of cats to adapt to sequences of perturbations following the denervation of the PB and ST muscles. The important observation from these experiments was that, although short-term increases in stepping height were evident after a sequence of perturbed steps, these increases did not persist. This can be seen by comparing the increases in step height for cat 1 when intact (Figure 4.2) and following the denervation of PB and ST (Figure 4.8). Note that the increase in step height persists for 24 hours in the former case, while disappearing after five minutes in the latter case. Similar differences were evident for cat 3. A previous study (Tachibana et al. 2006) presented evidence that, in cats lacking the main knee flexors, MG and LG (normally and ankle extensors) may be recruited to flex the knee. However, the activity in MG was not sustained, and the step height dropped back to control values quickly.

4.3.4 Forebrain regions are required to encode and express the persistent hyperflexion

As shown in Figure 4.7, most cats expressed the increased step height only in the context in which they encountered perturbations, and Figure 4.5 shows examples of this recognition of environmental cues related to context. This implies that forebrain regions, especially visual and auditory regions of the cortex are involved in the expression of the persistent hyperflexion. We predicted, therefore, that the persistent hyperflexion would not be evident in decerebrate walking animals. Figure 4.9 shows that this was the case. Although a transient adaptation was evident, it lasted for only short periods, as the progressively decreasing step height in Figure 4.9A shows. Figure 4.9B shows averaged data from two animals, one of which had very small increases in step height, while the other had significant but short-lived increases in step height.

We also tested, in one cat, whether the conditioned hyperflexion in the intact animal persisted following decerebration. If it did then this would have been strong evidence that the adaptive changes described in this study involved changes in the cerebellum, brain stem and/or spinal regions. However, no increase in step height (as compared to the leg which did not encounter perturbations while intact) was evident in the decerebrate state, nor was there any difference in the response to multiple perturbations, compared to animals that had not developed adaptive responses while intact (data not shown).

4.1 Discussion

4.1.1 Characteristics of persistent hyperflexion

Locomotor systems adapt to changes of the musculoskeletal system or the environment to maintain stable and efficient movement (Emken and Reinkensmeyer 2005, Jensen et al. 1998, Lam et al. 2006, Rossignol et al. 2004, Tachibana et al. 2006). In this study we have shown that following a small number of mechanical stimuli to the paw of walking cats during the swing phase, there is a significant increase of the maximum step height of the perturbed leg. Figure 4.2 shows that this effect is persistent, lasting for at least 24 hours in two animals in the absence of further stimuli. In the remaining three animals, the maximum step height was elevated 24 hours later but this increase was not statistically significant (not shown). However, the stumbling-corrective response was enhanced even in the absence of persistent hyperflexion (Figure 4.4). Figure 4.3 shows that an increase in knee flexion was primarily responsible for the increase in step height, although ankle flexion also increased in one animal. This increase in knee flexion is similar to previously described flexion responses when cats encounter obstacles during swing (Buford and Smith 1993). Increases in the activity of the ST muscle (Figure 4.5), as well as the absence of the persistent hyperflexion in animals following the denervation of PB and ST, suggest that these primary knee flexors are an important part of the hyperflexion. We also examined the activity of IP (a hip flexor) and Sart (a hip/knee flexor) in two animals and saw no significant increase (data not shown). One clear feature of this adaptation is that it does not generalize to all environments. Figure 4.6 shows that maximum step height returned to normal when the cat was placed

in a new environment, but once again increased when returned to the environment in which it had experienced the stimuli while walking. In some cases, an increase in step height in response to a visual or auditory cue that a cat associated with perturbed steps was very striking (Figure 4.7A). In addition to increasing maximum step height during all steps, cats also developed an increased response to additional perturbations while walking. Figure 4.4 shows that the steps following contact with an obstacle during swing remained much higher in animals that had repeatedly been perturbed while walking, even after the persistent hyperflexion had waned. In one intriguing case, the contact with an object by the opposite leg, which had a normal stepping height and flexion response, led to a sudden and marked increase in stepping height in the leg which had developed persistent hyperflexion (Figure 4.4B). This suggests that the enhanced flexion response may reflect the initiation or enhancement of a context-dependent locomotor adaptation, rather than an alteration of the flexion response itself. Contact with an obstacle by *either* leg would provide additional sensory cues that would reinforce the recognition of the context.

4.2.2 Comparison of durations of the persistent hyperflexion and other forms of adaptation

One unusual aspect of the persistent hyperflexion we describe was its duration. Figure 4.4 shows that the adaptation persisted for up to three days in one animal, in the absence of any further stimuli. After-effects have been described previously for forms of locomotor adaptation but they persist for much less time. For example, when stepping in an artificial force field, humans quickly adapt to overcome an initial change in step

height. After the removal of the force field, there is a brief aftereffect which decays over the course of three to five steps (Emken and Reinkensmeyer 2005). Similarly, humans adapted to walking in a robotic device which alters the torques necessary to flex the joints of the leg display an aftereffect which lasts on the order of tens of steps when the device is shut off (Lam et al. 2006). An important difference between these forms of adaptation and the persistent hyperflexion we describe in this paper is that the former involve the modification of the dynamic properties of the leg; that is, they change the movement that results from a given amount of muscle activity. This form of adaptation has been widely studied in the context of reaching movement, and has been hypothesized to involve modifications of an internal model of limb dynamics (Kawato 1999, Lackner and DiZio 2005, Ostry and Feldman 2003, Wolpert and Ghahramani 2000). These modifications could be based on a difference between expected and actual movements, caused by the unusual limb dynamics (Wolpert and Ghahramani 2000). Adaptations to walking on rotating disks (Gordon et al. 1995) and split-belt treadmills (Jensen et al. 1998), which also display short-lived after-effects, would similarly involve a discrepancy between the actual and the expected movement. This process would be equally active once the normal limb dynamics are restored (during which expected and actual movements would again be different), leading to a rapidly declining aftereffect. Figure 4.10 highlights an important difference between this form of adaptation and the one we describe in this paper. We did not change the dynamics of the limb – rather, we introduced a change to the environment. When this change was removed, there was no positive signal that the environment has returned to normal, as there is when an artificial force field is removed or split-belt treadmills are returned to the same speed. We hypothesize the lack of a

positive signal that the environmental change has ceased is a crucial feature in the persistence of this hyperflexion.

4.2.3 Functional relevance of persistent hyperflexion

This distinction between adapting to altered limb dynamics and adapting to an environmental change may also be relevant when considering the functional relevance of the persistent hyperflexion (Figure 4.10). A number of studies have shown that the basic pattern of walking is altered in functionally relevant ways in certain contexts with particular environmental challenges. For example when cats walk up (Carlson-Kuhta et al. 1998) or down (Smith et al. 1998b) inclines, there are changes to the flexor and extensor synergies that are normally evident during level walking. A second example is that when adult humans walk on a compliant surface, there are changes in muscle activity, step height, and center of mass height when compared to walking on a solid surface (Maclellan and Patla 2006). It is possible that the particular patterns of locomotor activity that are needed to negotiate these situations are learned early in life, and are then expressed as needed within the correct context. An example can be seen in adult humans walk onto a moving platform which can slide forward. Subjects in this situation develop an adaptive response to minimize the resulting sway (Reynolds and Bronstein 2003), and this adaptation shows only limited expression in other contexts (Reynolds and Bronstein 2004). These modifications maintain stability and efficiency in the particular environment where they are expressed. They are unlikely to be induced by immediate feedback from the environment, because they involve major changes in the timing of muscle activity (such as the appearance of flexor bursts during the stance phase of

walking (Smith et al. 1998b)) and they persist in the absence of the perturbing stimuli (Reynolds and Bronstein 2004). We believe that the persistent hyperflexion that we describe in this study reflects a similar, context-dependent locomotor program which minimizes the effect of repeated mechanical stimuli during walking in one environment. We predict that locomotor adaptations which involve discrepancies between expected and actual movements would be much more generalized, as is the case in response to walking on a rotating disk (Gordon et al. 1995). Although we do not know of any similar studies that have specifically tested the generalization of adaptation, it is relevant that adaptation of human reaching movements to an imposed force field is not restricted to environments with identical visual cues, such as room lighting color (Gandolfo et al. 1996).

4.2.4 Site of locomotor adaptation

The persistence and the context-dependent nature of the locomotor adaptation we describe in this study motivated us to try to gain insight into the neural structures that are involved. Locomotor output is the product of complex interactions of many areas of the nervous system, including spinal pattern generators (Dietz 2003, Rossignol and Dubuc 1994), muscle afferents (Dietz and Duysens 2000, Pearson 2004) and the forebrain (Drew et al. 1996, Drew et al. 2002). One theme which has emerged from the study of locomotor adaptation is that many regions of the nervous system-are involved in the process of locomotor adaptation (Bouyer et al. 2001, Carrier et al. 1997, Chen and Wolpaw 2005, Whelan and Pearson 1997, Wolpaw and Tennissen 2001).

Nonetheless, a number of studies have shown that spinal circuits alone are able to learn new locomotor behaviour. Clear demonstration of this are that spinalized cats can

recover stepping after training (Barbeau and Rossignol 1987), both spinalized cats (Edgerton et al. 2004) and decerebrate ferrets (Lou and Bloedel 1988a) increase flexion to avoid obstacles in the environment, and spinalized rats decrease stance duration to accommodate a force field applied to the leg (Timoszyk et al. 2002). However, we do not believe that spinal circuits are the site of long-lasting changes during the development of persistent hyperflexion, because one clear aspect of this adaptation is the dependence on context (Figure 4.6) which implicates descending signals from cortical regions of the brain. Additional evidence comes from an experiment in which we performed a decerebration on one animal that displayed persistent hyperflexion after repeated perturbed steps in the intact state. If, in the decerebrate state, the stepping of the leg that had previously been perturbed was markedly different from the contralateral leg, this would suggest that the persistent hyperflexion involved changes in the spinal circuits controlling stepping. This is a similar approach to that taken by (Carrier et al. 1997). However, we saw no relevant differences in this animal (data not shown), although further study is necessary before we conclusively say whether changes at the spinal level are not involved in the expression of persistent hyperflexion. For example, it may be that adaptive changes are manifested at the spinal level, but are not observed in the absence of appropriate descending commands from the forebrain.

As spinal circuits are insufficient for both the expression (Figure 4.6; discussion above) and the acquisition (Figure 4.9) of persistent hyperflexion, we believe that supraspinal regions are an important component of the development of this adaptive behaviour. It is likely that the cerebellum is necessary, as earlier studies implicate the cerebellum in other locomotor adaptations, such as adapting to changing treadmill speeds

(Rand et al. 1998, Yanagihara and Kondo 1996), rotating disks (Earhart et al. 2002) and environmental perturbations (Lou and Bloedel 1986, Lou and Bloedel 1988b). Additional inputs certainly come from visual, auditory and integrative regions of the cortex as an important feature of the persistent hyperflexion is that its expression is dependent on context. This implies that forebrain regions must be involved in recognizing the visual or auditory cues associated with this context, and subsequently lead to the initiation of hyperflexion. In one animal, the speed of walking was also a factor in the expression of hyperflexion (Figure 4.5). We cannot say whether proprioceptive feedback about the speed of walking, or a central signal related to walking speed (Jordan 1998) is responsible for this specificity.

While it is likely that associative cortices are crucial for recognizing the contextual cues, the motor cortex is a strong candidate to initiate and maintain the hyperflexion once these cues are recognized. Lesion studies (Drew et al. 1996, Drew et al. 2002) as well as single-unit recordings (Widajewicz et al. 1994) show activity of the motor cortex to be important for increasing flexion of the hindlimb to negotiate obstacles. Furthermore, recent studies have shown that microstimulation of the motor cortex enhances ST activity and increases joint flexion (Bretzner and Drew 2005a), as well as increases the strength of cutaneous reflexes (Bretzner and Drew 2005b). These are all features of the hyperflexion behavior described in this paper (Figures 4.3, 4.6, 4.7). One intriguing finding from the current study was that the denervation of the knee flexors PB/ST eliminates the long-lasting nature of the hyperflexion, although short-term increases in step height remain (Figure 4.8). We believe that these short-term increases may be partially due to compensatory activity in LG and MG muscles, which cross both

the knee and the ankle joint (Tachibana et al. 2006). However, the absence of long-lasting hyperflexion is consistent with the hypothesis that activity from the motor cortex aimed at increasing activity in knee flexors is an important component of the persistent, context-dependent adaptation. Lesioning (Drew et al. 2002) or temporary inactivation (Drew et al. 1996) of the motor cortex in animals that had previously developed persistent hyperflexion would be one useful technique to evaluate this hypothesis. Single-unit recordings of the motor cortex before, during and after persistent hyperflexion (Widajewicz et al. 1994) would also help to confirm or deny this hypothesis.

4.6 Figures

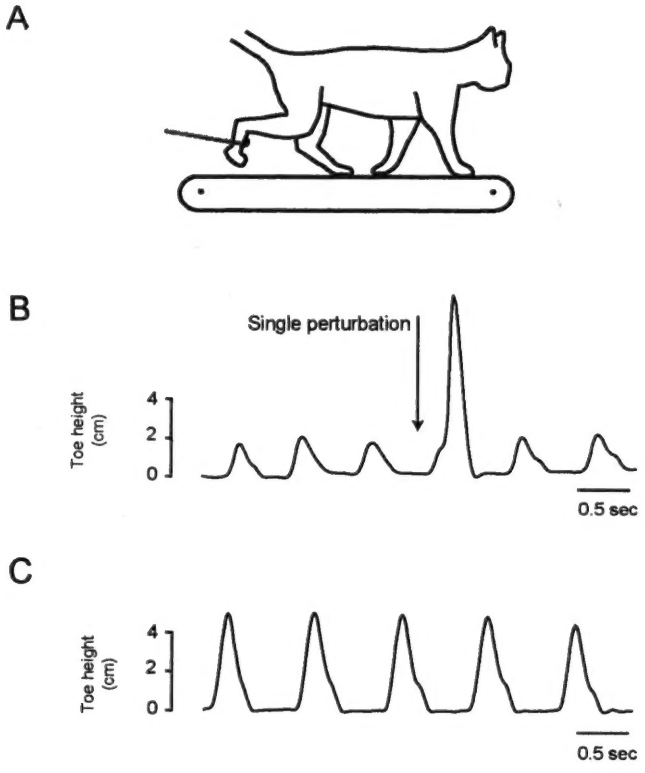

Figure 4.1

Stumbling-corrective response and persistent hyperflexion in the walking cat.

A) The method used to perturb the hindleg of walking cats. A handheld, padded obstacle was placed in front of the dorsum of the paw during each step.

B) Stumbling-corrective response in normal animal (cat 4) before repeated perturbations. After contacting an obstacle during the swing phase of locomotion (indicated by arrow), the toe is lifted quickly over and placed on the opposite side, where normal stepping resumes.

C) Increased step height (persistent hyperflexion) is evident following 120 perturbed steps in cat 4. Note the increase when compared to normal step height in the same animal, before perturbed steps, in B.

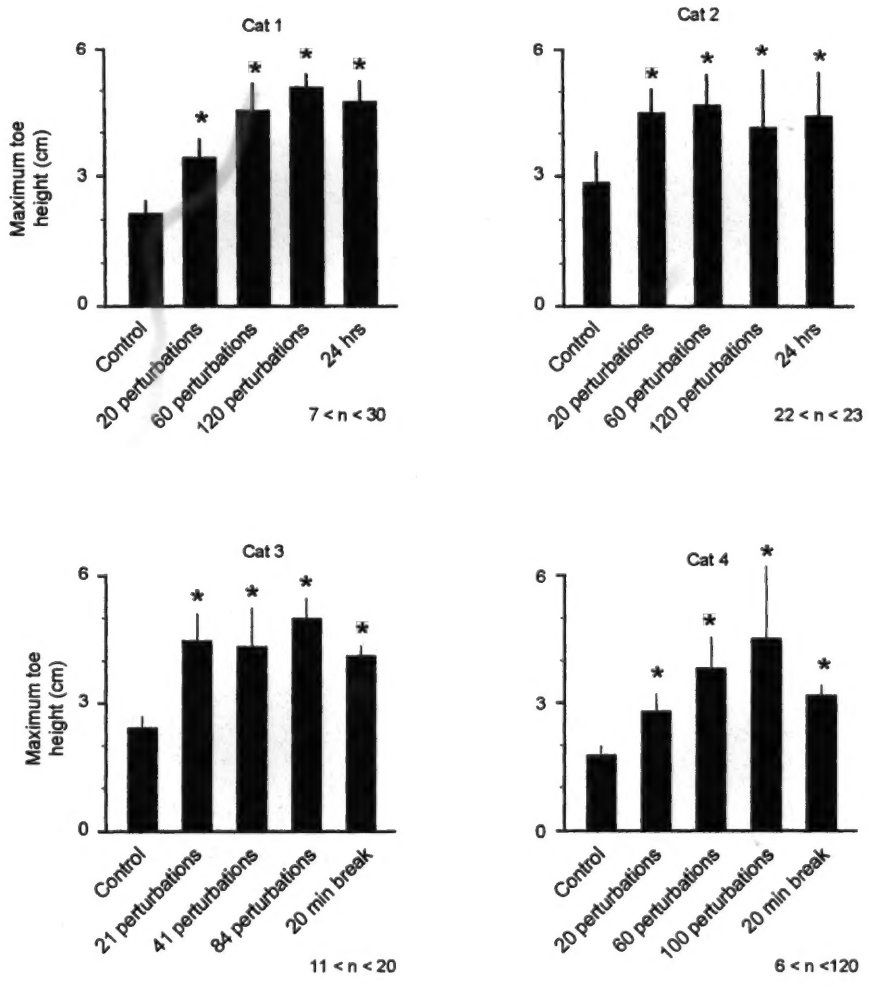

Figure 4.2

Bar graphs showing mean maximum step height following sequences of continually perturbed steps and extended delay periods (24 hrs, cats 1,2; 20 min, cats 3,4) for four cats. Data is taken from steps immediately following conditions described on abscissa. Asterisks denote significant difference from control step height ($p < 0.05$, Kruskal-Wallis test). Error bars denote one standard deviation.

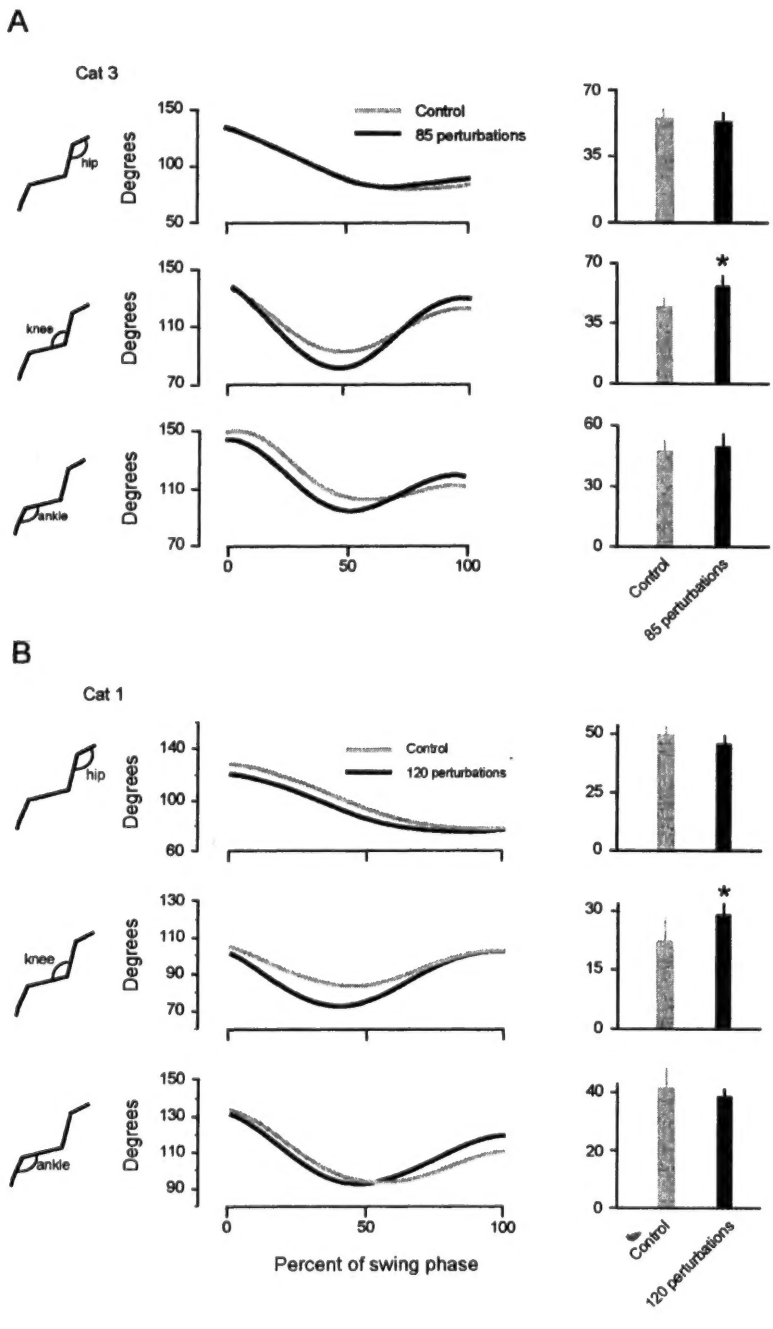

Figure 4.3

Increase in step height is primarily due to an increase in knee flexion shown in 2 cats. Left column shows mean joint angles through the swing phase of stepping, before (gray lines) and after (black lines) repeated perturbations ($7 < n < 17$). Step durations were normalized and aligned on the initiation of swing prior to averaging. Right column shows mean flexion of each joint (angle at peak flexion minus angle at initiation of swing) before (gray bars) and after (black bars) repeated perturbations ($7 < n < 17$). Asterisks denote significant difference ($p < 0.05$, one-tailed t-test). Error bars represent one standard deviation.

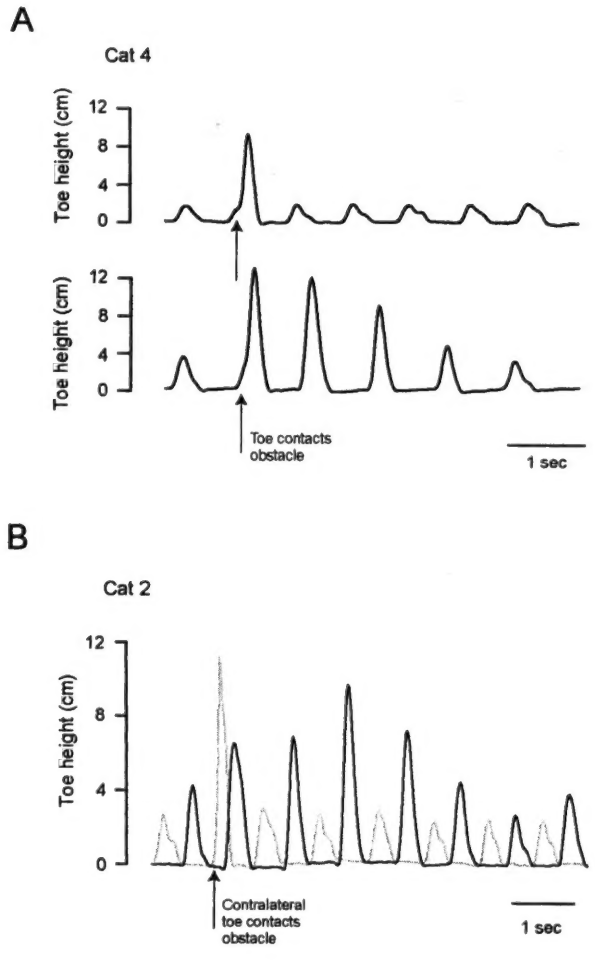

Figure 4.4

The stumbling-corrective response is enhanced even in the absence of persistent hyperflexion. A) Representative steps from cat 4 show the response to a single perturbation after a sequence of continually perturbed steps (in this case, twenty minutes later, after persistence hyperflexion had waned – lower panel) led to an increase in step height for a number of steps which is not present in the normal stumbling-corrective response (upper panel). Perturbed step is denoted by an arrow. B) In one animal, evoking a stumbling-corrective response in the opposite leg let to an increase in step height in the leg that had experienced multiple perturbations. Black line shows the step height of the ipsilateral (persistent hyperflexion) limb, while gray line shows the step height of the contralateral limb. Perturbed step of contralateral leg is denoted by an arrow.

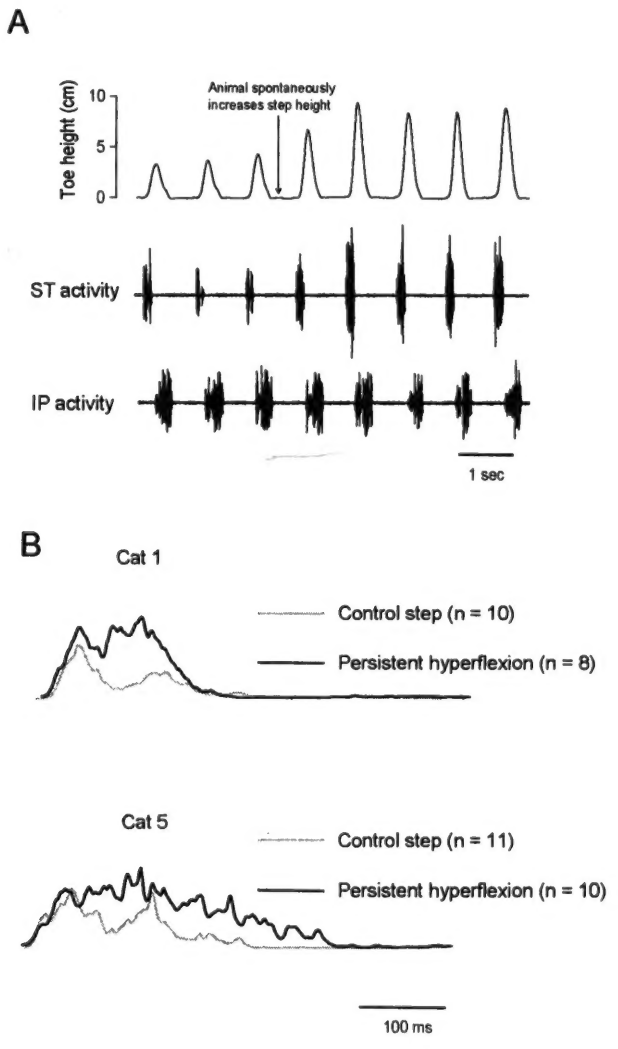

Figure 4.5

Increase in step height is accompanied by increases in ST activity. A) Data from cat 1 showing the same sequence of steps as in Figure 4.7A (lower panel). Step height is shown above unrectified EMG traces from IP and ST muscles. Note the increase in ST activity accompanies the increase in step height, while IP is unchanged. B) Mean ST activity from cats 1 and 5 shows increased magnitude and duration of activity. Gray traces show ST activity during control steps, while black traces show ST activity after the development of persistent hyperflexion. Bursts are aligned on the initiation of ST activity.

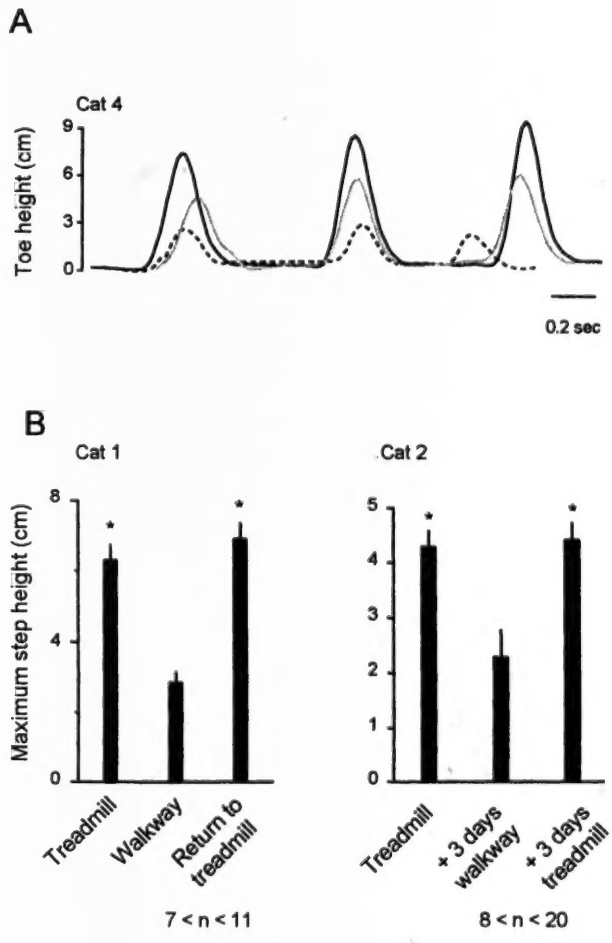

Figure 4.6

Increase in step height was restricted to the context in which perturbed steps were experienced. A) Representative examples from cat 4 show step height after a sequence of perturbed steps on the treadmill (gray line), upon transfer to walkway (dotted black line), and upon return to treadmill (solid black line). B) Mean step height for cat 1 and 2 following a sequence of perturbed steps, in the walkway, and upon return to the treadmill. Note that the increase in step height persisted for three days in cat 2. Asterisks denote significant difference from step height on walkway ($p < 0.05$, Kruskal-Wallis test). Error bars represent one standard deviation.

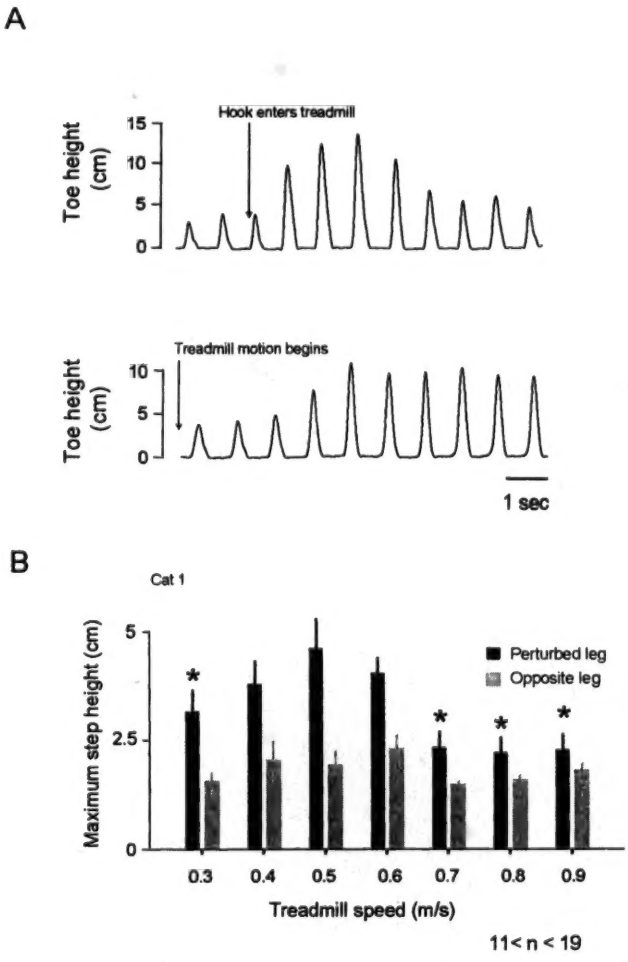

Figure 4.7

Sensory cues can evoke expression of persistent hyperflexion. A) Top panel shows step height from a sequence of steps from cat 2. Step height increases when the hand-held obstacle enters the treadmill, although no cutaneous stimuli was experienced. Lower panel shows a gradual increase in step height in cat 1 after the treadmill motion begins. Again, no cutaneous stimulation was experienced.

B) Step height in cat 1 was maximum at the treadmill speed at which perturbations had been experienced (0.5 m/s). All data are from steps after a sequence of perturbations had led to an increase in step height. Black bars – perturbed leg; gray bars – opposite leg. Asterisks denote significant difference from step height at 0.5 m/s. Error bars represent one standard deviation. Note that opposite leg shows little change in step height at different speeds.

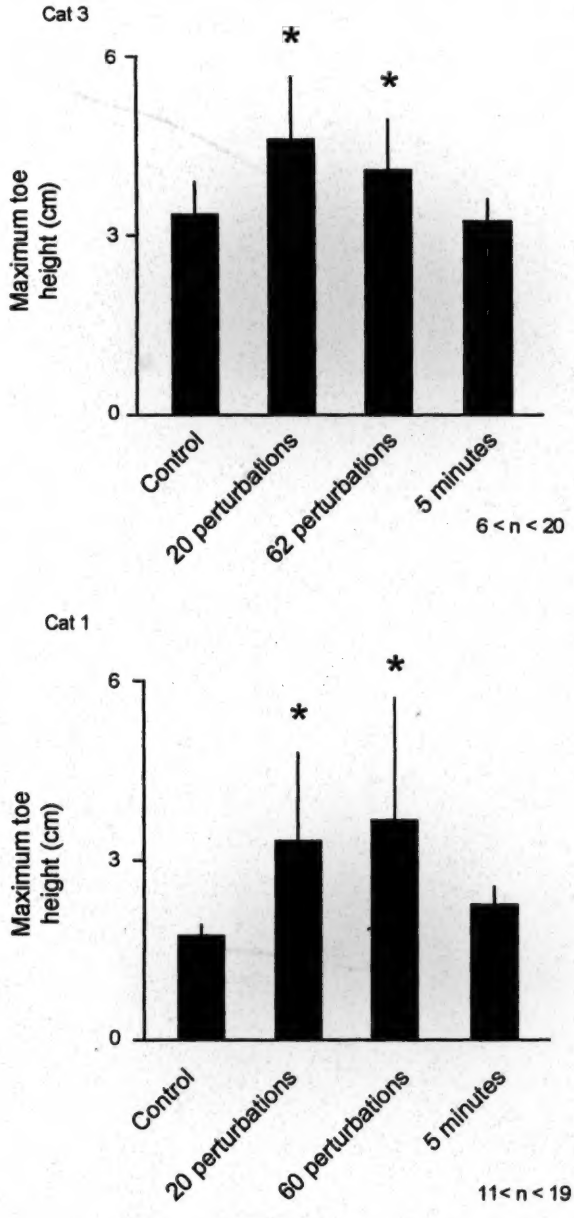

Figure 4.8

Denervation of knee flexors prevented long-term increase in step height, although short duration increases remains. Mean step height following sequences of perturbed steps in cats 3 and 1 following denervation of knee flexors PB and ST. Asterisks denote significant difference from control step height ($p < 0.05$, Kruskal-Wallis test). Error bars represent one standard deviation.

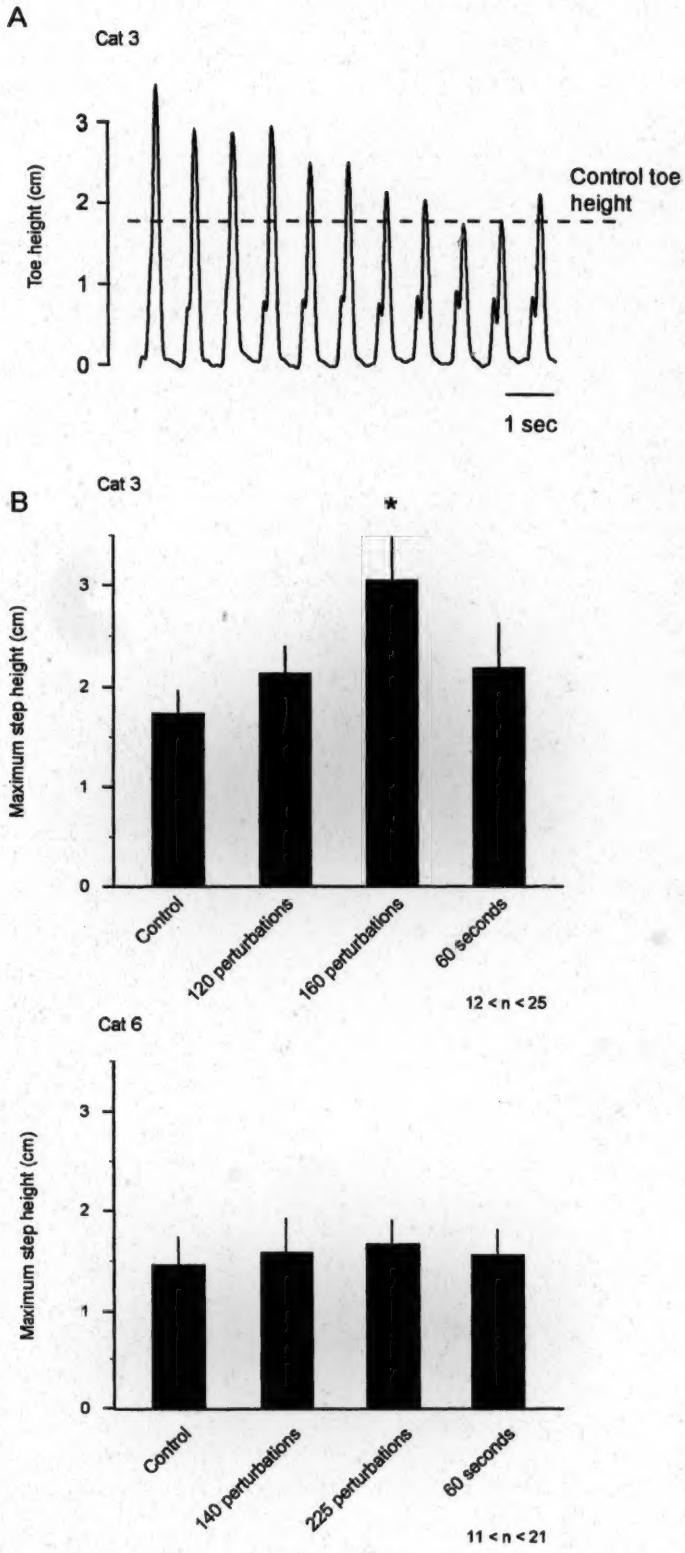

Figure 4.9

Decerebrate animals show no long-term increase in step height following a sequence of perturbed steps. A) A series of steps immediately following 80 perturbed steps showing a transient increase in height which decays quickly. B) Mean maximum step height in two decerebrate cats. Mean values are of steps immediately following the condition described on the abscissa. Asterisks denote significant difference in step height from control ($p < 0.05$, Kruskal-Wallis test). Error bars represent one standard deviation.

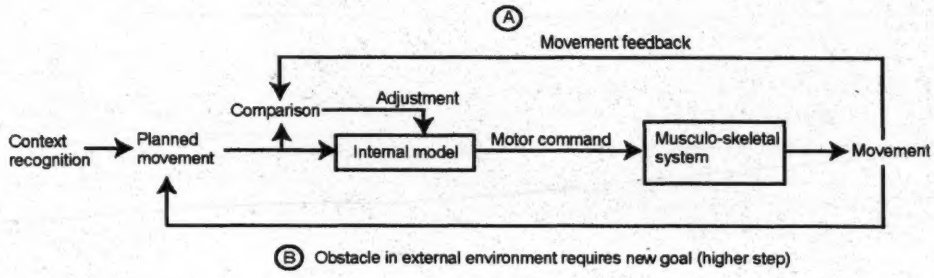

Figure 4.10

Conceptual model showing two potentials forms of locomotor adaptation. In A), discrepancies between the goal and the actual movement (reported via afferent feedback) lead to changes in the internal model of limb dynamics to eliminate or reduce the discrepancy. In B), recognition of a particular context results in a goal specific to that context (in this case, stepping high to avoid obstacle). This form of adaptation would be longer lasting, because there is no discrepancy between expected and actual movements when the obstacle is removed, and less generalized, because it is expressed only when the appropriate context is recognized.

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Chapter 5[5]

5.1 Introduction

This thesis presents studies of three forms of flexibility in the walking system of the cat. In Chapter 2, the regulation of the swing-to-stance transition during walking in variable environments was studied. The main finding was that the degree of flexion of the hip influences this transition, helping to maintain stable locomotion. In Chapter 3, the role of memory during visually-guided stepping was examined. The main finding was that long-lasting and precise memories guide the movement of the hindlegs of cats over obstacles. In Chapter 4, a long-lasting and context-dependent increase in step height following repeated perturbed steps was studied.

This section of the thesis contains further discussion of the issues raised by Chapters 3 and 4. It begins with a comparison of the role of vision and memory in human and cat locomotion. It then considers which areas of the nervous system could be involved in the long-lasting memories described in Chapter 3. Finally, it discusses which structures of the nervous system could be involved in the acquisition and expression of the locomotor adaptations described in Chapter 4, expanding on what was presented in that chapter to make the distinction between sites required for the *acquisition* and the *expression* of locomotor adaptation.

5.2 The use of visual information during human and cat locomotion

Personal experience tells us that visual information is used for many purposes during locomotion. Consider walking a four or five block journey from your front door to

[5] A version of this chapter has been accepted for publication:
Progress in Motor Control V, Sternad D & Nichols TD, *eds.*

the nearest bus stop. Orienting your body correctly, walking in the correct direction, making turns when necessary, stepping up and over curbs, and crossing streets safely all rely on visual information (Patla 1997). Vision has other roles during locomotion which are not as obvious to introspection. For example, the movement of the visual field as we move (called *optic flow* – see (Gibson 1958, Sherk and Fowler 2001a)) provides useful information about the speed and direction of walking.

5.2.1 Intermittent visual input is sufficient to guide foot placement during constrained walking

When the feet have to be precisely placed during walking (as would occur when walking on a narrow ledge), there is considerably more need for visual feedback. One consistent theme emerging from a number of different studies is that visual information is used to plan and initiate steps (called *feedforward control*) rather than being used to monitor and correct ongoing leg or foot movements (called *feedback control*). Early evidence of this came by examining volunteers as they walked towards a target on which they had to precisely place their feet. The lighting conditions could be varied using intermittent flashing lights. Three hundred millisecond flashes of illumination once per step were sufficient to reach the target without difficulty, even though there was no visual feedback in between flashes (Laurent and Thomson 1988). Similarly, when volunteers used the glasses described above after being asked to step in specific positions while walking, they accessed visual information during thirty-five percent of walking time (Patla et al. 1996). Although infrequent, this is three times as often as during unobstructed walking. This intermittent sampling is used to establish a memory of the

required step positions which can be used when the glasses are translucent and vision is not available. Recently, a similar experiment was repeated except that investigators recorded gaze direction (Patla and Vickers 2003). This revealed that, even when stepping on irregularly spaced markers, much of the time walking is spent looking ahead at a spot that moves along with walking movements (termed *travel fixation* by the authors). When volunteers did fixate a required foot position, it was usually a position two or more steps ahead (Patla and Vickers 2003).

A situation in which continual visual feedback might be required is as people walk up to and step over an obstacle. Again, however, a number of studies have shown that visual information is used to plan necessary movements in advance, and not to adjust the movements of the legs as they step over obstacles. One line of evidence comes from recording where people look when the walk up to and over obstacles. Using a helmet that could track the direction of gaze, investigators found that human subjects fixate obstacles as they approach them, but not during the step before or over them (Patla and Vickers 1997). Instead, they spend much of their time looking ahead at a point that moves as they walk forward (travel fixation). Similarly, when volunteers wearing translucent/opaque glasses as described above walked up to and over obstacles, they voluntarily accessed visual feedback only intermittently. Importantly, this sampling of visual feedback came much more frequently during the steps leading up to the obstacle than to during the step over the obstacle. Furthermore, increasing the height of the obstacle resulted in volunteers accessing more visual feedback, and for longer, during the approach to the obstacle, but did not change the amount of visual feedback accessed during the step over the obstacle (Patla et al. 1996). This suggests that visual information about the obstacle

gathered in the steps leading up to the obstacle, but not during the step over the obstacle (Patla 1997). Consistent with this conclusion, volunteers who had visual input removed (by dimming illumination) during the last step before stepping over an obstacle stepped as accurately as when their vision was not blocked (Patla 1998).

5.2.2 Dynamic visual input can be stored in memory and used to guide leg movements

As discussed above, multiple lines of evidence have shown that the necessary visual information to guide leg movements is collected a few steps before it is needed. Can obstacles be successfully stepped over using visual information collected even earlier? This question was tested by having subjects fixate an obstacle for 1.5 s while standing five steps away, and then having them walk towards it (Patla 1998). Volunteers successfully stepped over the obstacle with the lead (first) limb about 75 percent of the time. This performance improved to 90 percent success when volunteers started eight steps away, and walked forward for about three steps before having visual information removed. A more recent study confirmed that visual feedback obtained while walking towards an obstacle (called *dynamic visual feedback*) leads to much more accurate steps over the obstacle compared to visual feedback while standing still (Patla and Greig 2006). The authors also showed that the benefits of the dynamic visual feedback are not obtained unless locomotion is continuous towards the obstacle – that is, walking backwards away from the obstacle, or walking towards it, stopping, and restarting, resulted in performance that was no better than viewing the obstacle while standing still. The authors also noted that it was the position of the feet in the step before the obstacle, and not the height of the obstacle, that led to collisions. From these observations, they concluded that the visual

feedback obtained as the image of the obstacle moved across the retina during forward walking is an important aspect of determining the location of the obstacle relative to the observer.

5.2 Visual-guidance and memory during stepping in quadrupeds

Like humans, cats often maintain a constant gaze angle while walking, resulting in the same travel fixation pattern as humans (Fowler and Sherk 2003). Cats can rely on visual memory to step accurately for three to four seconds in the absence of visual feedback (Wilkinson and Sherk 2005) and dynamic feedback obtained while moving is important to the establishment of this memory, similar to in humans (Sherk and Fowler 2001b).

5.2.1 Cats have uniquely long-lasting memory which guides the hindlegs during visually-guided stepping

One important difference between visually-guided stepping in cats and human is that cats, like all quadrupeds, have an unavoidable need for visual memory as they step over obstacles. This is because the control of the hindlegs over an obstacle cannot be guided directly by visual information, as the head and eyes have passed over the obstacle by the time the hindlegs step over it. This provides a unique model to study the role of memory during walking which is the basis for Chapter 5 of this thesis.

5.2.2 Movement of forelegs may be an important signal during visually-guided movement of hindlegs in cats

One key difference between the long-lasting visual memories we describe in cats (Chapter 5 of this thesis, (McVea and Pearson 2006)) and previously described short-lived memories (4-5 seconds during locomotion (Wilkinson and Sherk 2005); 30 seconds during delayed searching task (Fiset and Dore 2005)) is that cats straddling an obstacle have interacted with the obstacle – they have stepped over it with the forelegs. Thus, it is reasonable to predict that a crucial component of the long-lasting activity which guides the hindlegs may be signals related to the movement of the *forelegs* over the obstacle. To test whether signals related to movement of the forelegs is a contributor to the neural activity that persists to guide the hindlegs even during long delays, we repeated the experiments described in Chapter 5, except that cats were stopped for the delay before they had stepped over the obstacle at all. Figure 5.1 shows that, in cases where the forelegs did not step over the obstacle, there was an increase in step height following only short delays. Furthermore, this increase was small and was often absent even during short delays. When the forelegs *did* step over the block, the movements of hindlegs revealed a memory that lasted for minutes. In both instances, other factors such as the visual input and the walk towards the obstacle were identical.

5.3 Putative role for the parietal cortex in visuomotor transformations during locomotion

The visual-guidance of locomotion requires a flow of information from areas of the brain which receives only visual inputs (such as the lateral-suprasylvian cortex in the cat) to areas which coordinate motor output (such as the motor cortex). These extreme ends of this circuit are primarily involved with motor or sensory activity, but not both. At

the sensory end, lesions in LS, for example, do not produce any significant motor deficits during unobstructed walking (Sherk and Fowler 2002); at the motor end, activity in the motor cortex and corticospinal neurons do not increase their activity when an obstacle is in view, but only at the time of the necessary motor adjustment (Drew 1991). However, important processes are involved in between the sensory and motor areas. For example, behavioral observations show that humans (Patla 1997, Patla 1998, Patla and Greig 2006) and cats (McVea and Pearson 2006, Wilkinson and Sherk 2005) do not need constant visual feedback for visually-guided stepping. This suggests that neural areas related to visual memory can provide input to motor areas when needed. Other, possibly overlapping, areas must be involved in the transformation of signals from a sensory frame of reference to a motor frame of reference. Such a transformation is an essential component of any movement that is guided by sensory information, such as picking up a coffee cup. To make an appropriate reach for the cup, we need to know where it is relative to our hand, but the sensory information we have about it tells us where it is relative to our eyes. In a similar fashion, when we step over a tree branch in our path, we need to know where the branch is located relative to our feet, not our eyes or heads.

One area likely to be involved in this process is the parietal cortex, which connects through both inputs and outputs to visual, somatosensory, and motor cortical area. These regions of the brain have been extensively studied in primates and are consistently found to be related to movement planning and transformations between sensory and motor information (Andersen and Buneo 2002, Andersen and Buneo 2003, Buneo and Andersen 2005, Colby and Goldberg 1999, Kalaska et al. 1997). Of particular interest is area 5, a narrow band of cortex at the rostral edge of the parietal lobe of the

brain. Although most of what we know about area 5 relates to primates, it has properties that seem suited to the task of guiding the hindlegs of cats over obstacles. When monkeys are trained to make reaching movements to a specific target after a delay period, for example, neurons in area 5 remain active throughout the delay (Kalaska 1996). This activity is representative of the location of the target relative to the limb (Kalaska et al. 1997, Lacquaniti et al. 1995), which suggests the neurons could be involved in initiating movements around remembered objects. There is also good evidence that area 5 neurons integrate somatosensory and visual inputs to form a representation of the position of the limbs and body. When these two inputs are dissociated by showing monkeys a realistic looking fake arm, area 5 neurons are most active when the fake arm and the monkeys real arm are in similar positions (Graziano et al. 2000). Moving the real and the fake arm to different positions reduces their activity, suggesting that area 5 neurons combine visual and somatosensory inputs to represent body position (Graziano and Botvinick 2002).

5.3.1 Area 5 may be involved in controlling hindlegs during visually-guided stepping in the cat

One particular task in which the need for memory and sensorimotor transformations is evident is the memory-guided stepping over obstacles we describe above (McVea and Pearson 2006). In this case, visual information about the location of the obstacle must be integrated with information about the location of the hindlegs to form an accurate representation of the obstacle relative to the legs. This representation must be persistent and be updated as needed (if the cat moves slightly forward, for example) throughout the long delay periods we describe. Cells involved in this process

would need to integrate information about the visual scene, the position of the limbs, and the any movements of the animal, all factors that determine when and how the hindlegs must be lifted.

The properties of the neurons in area 5 – particularly their integration of inputs related to both movement targets and body position – are properties that would be necessary for cells guiding the hindlegs of walking cats over obstacles. Although we know less about area 5 in the cat than in primates, there is strong evidence to suggest it has roles similar to those described in the primate. Feline area 5 receives inputs from visual areas of the cortex(Avendano et al. 1988, Kawamura 1973), including LS (Avendano et al. 1985) (whose involvement in visually-guided locomotion is described above), the primary somatosensory cortex, the motor cortex (Avendano et al. 1985, Avendano et al. 1988), thalamic visual nuclei (Symonds et al. 1981), as well as from the cerebellum via the thalamus (Kakei and Shinoda 1990, Sasaki et al. 1972). Area 5 sends outputs to the motor cortex (Andujar and Drew 2005, Babb et al. 1984, Ghosh 1997, Kakei et al. 1996) and the cerebellum (Kakei et al. 1995) as well as other areas of cortex (Kawamura 1973). This rich collection of inputs and outputs suggests that area 5 might be an important connection between sensory and motor areas in the cat, as in primates (Beloozerova and Sirota 2003, Jiang and Drew 1996, Kakei et al. 1995). Investigators have begun to test this hypothesis by recording from and manipulating area 5 during motor tasks that require visual guidance. An early study showed that lesioning the suprasylvian cortex of cats (a region that includes area 5, as well as area 7 and 21) resulted in significantly poorer accuracy when reaching to a moving target (Fabre and Buser 1981). There was no decrease in performance in cats that received lesions to areas

7 and 21 only. Importantly, the loss of these cortical areas did not affect the reaction time or the speed of movements, only the accuracy of movements. Taken together, these results suggest that the transformation of visual data for use by motor areas is an important role of area 5.

5.3.2 Lesions to Area 5 impair visually-guided stepping

Recently, investigators have tested whether this is the case during visually-guided stepping movements as well as reaches. In a unique study, cats were trained to step over obstacles that moved at a different speed than the treadmill on which they were walking. Because the speed of the obstacle towards the cat was different than the speed of the cat itself, only visual information, and not feedback about the cat's motion, could be used to successfully avoid the obstacles. The posterior parietal cortex (a region that includes area 5 as well as area 7) was shown to be important for this process, as two of three cats that were subsequently lesioned in this region could not position their paws to correctly avoid the obstacle (Lajoie and Drew 2005).

5.3.3 Area 5 neurons activity is modulated during visually-guided stepping

Single-unit recordings made while cats perform challenging locomotor tasks have also supported the hypothesis that area 5 is important in the visual guidance of locomotion (Beloozerova and Sirota 2003). The first observation was that most cells recorded were rhythmically active during unobstructed walking, although, unlike in the motor cortex, many cells showed two peaks of activity per step. Because very few of the recorded cells responded to touch on the limbs of the cat, the authors concluded this

activity was likely to represent duplicate signals sent to area 5 as well as to the muscles from the motor cortex (this is termed *efference copy)* and not signals generated by touch sensors as the limbs moved. This connectivity to the motor cortex is consistent with our observations that movement of the forelegs over obstacles (and the accompanying increase in motor cortex activity) appear to be important in establishing long-lasting memories of obstacles (McVea and Pearson 2006). Many cells were also responsive to visual stimuli, as would be expected in an area involved in the coordination of visual inputs with motor outputs. When recordings were made during either walking on ladder rungs or walking over a series of obstacles, both tasks which require visual guidance, the activity of area 5 neurons changed substantially. Some neurons that were not active during normal locomotion became active, while others that changed their time of activity relative to stepping movements, or developed an additional peak of activity in each step.

What is the purpose of this altered activity? Beloozerova and Sirota (2003) concluded that the observed activity in area 5 is responsible for the increased activity in the motor cortex during visually-guided locomotion. Instead, they concluded the output from area 5 may act directly on spinal interneurons or subcortical motor areas to produce modifications of locomotion. They make this conclusion because the timing of activity of neurons in the motor cortex is relatively unchanged during visually-guided walking, whereas many neurons of area 5 shift their timing or develop additional periods of activity. However, the authors did not consider that additional peaks that appear in area 5 neurons when vision is required may be related to the coordination of the forelegs and hindlimbs – they consider this activity only in relation to the ipsilateral forelimb. In fact, motor cortical cells related to the movement of the hindlegs have been described that do

change the timing of their activity during stepping over obstacles (see Figure 11, (Widajewicz et al. 1994)) exhibiting activity between the time that that foreleg touches down and the hindleg lifts off. This activity is not present during unobstructed walking. Given that area 5 has been shown to encode the location of reaching targets in primates (Kalaska 1996, Kalaska and Crammond 1995, Scott et al. 1997), it is a reasonable prediction that multiple peaks of activity in area 5 neurons may contribute to the activity of those motor cortex neurons that remain active between fore- and hindleg stepping and thus the coordination of leg movements over an obstacle. One way to test this would be to combine recordings of area 5 with the delayed obstacle-crossing test we describe above (McVea and Pearson 2006). If activity in area 5 neurons is sustained for the duration of the delay while the animal straddles the position of the obstacle, this would be strong evidence that the activity is related to the presence of the obstacle and the necessary step modification in the hindlimb. Preliminary recordings show this is a possibility in at least some cells (T Drew, personal communication).

5.3.4 Important visual inputs to area 5 may come from the cerebellum

Interestingly, area 5 cells that were visually responsive during rest were less responsive during visually demanding tasks, and those that were unresponsive at rest were more active during such tasks (Beloozerova and Sirota 2003). The authors suggested this was evidence that the parietal cortex received visual inputs that had already been processed or gated. One possible source of such visual input is the cerebellum. The lateral cerebellum receives visual inputs via the cerebropontocerebellar pathway (Robinson et al. 1984)and connects to the motor cortex via the ventrolateral

thalamus (Armstrong and Marple-Horvat 1996, Drew et al. 1996). Disrupting this connection by lesioning this thalamic nucleus decreases stepping accuracy during a ladder-walking task (Beloozerova and Sirota 1986) suggesting this circuit is important during visual guidance of locomotion. In an interesting experiment, both Purkinje and dentate neurons of the cerebellar hemisphere have been shown to rapidly react to visual stimuli during walking. In these experiments, the activity of cerebellar cells was recorded while cats walking across ladder rungs. One of the rungs could be unexpectedly lifted by up to 6 cm while cats approached it, providing a potent visual stimulus relevant to locomotion. Cerebellar cells responded strongly to this stimulus (more strongly than to other visual stimuli such as flash of light), and the response was stronger when the animal was closer to the lifting rung than when it was further (Marple-Horvat et al. 1998). Further study showed close associations between rhythmic activity in the lateral cerebellum and the motor cortex during visually guided stepping (Marple-Horvat and Criado 1999). Therefore, it is likely that the visual information for the modification of stepping arrives at the motor cortex both from cortical areas (such as LS) via corticocortical projections, and other areas (such as the cerebellum) via the thalamus (Drew et al. 1996).

5.4 Structures involved in the acquisition and expression of persistent high-stepping

A major aim of ongoing research is determine the relative roles of these different structures of the nervous system during locomotor adaptation (Carrier et al. 1997, Earhart et al. 2002, Pearson 2001, Whelan and Pearson 1997). One theme that has emerged is that it is necessary to differentiate between structures that are essential for the *acquisition* and

the *expression* of a locomotor adaptation (Bouyer et al. 2001, Carrier et al. 1997, Chen and Wolpaw 2005, Whelan and Pearson 1997, Wolpaw and Tennissen 2001). Chapter 4 of this thesis presents a long-lasting and contex-dependent form of locomotor adaptation in the walking cat which is relevant to this ongoing discussion.

5.4.1 Afferent feedback is important for acquisition of adaptive responses to perturbations

We first consider sites that are likely to be involved in the *acquisition* of the locomotor adaptation we describe in Chapter 4. One essential step in this process is sensing the perturbations during stepping and recognizing their persistence. Recently, strong evidence that the stumbling corrective response is initiated by low-threshold cutaneous receptors on the dorsum of the foot has been presented (Quevedo et al. 2005). This is consistent with conclusions from earlier studies (Prochazka et al. 1979, Wand et al. 1980). However, proprioceptive feedback from muscle afferents has also been shown to be important in the long-term adaptation of locomotor patterns to injury (Pearson et al. 1999, Pearson et al. 2003) and environmental changes (rotating disk) (Earhart et al. 2004, Jurgens et al. 1999). We cannot say what the relative contributions of cutaneous and proprioceptive feedback are to the development of the persistent hyperflexion that we describe. One worthwhile experiment aimed at this question would be to repeat our repeated perturbation protocol on animals that have had the cutaneous receptors on the dorsum rendered inactive via anesthesia. In this case, the stumbling corrective response is markedly reduced (Wand et al. 1980). If muscle afferents play an important role in the development of long-term hyperflexion, we would expect that although the initial

response to perturbations during stepping would be reduced, long-term adaptation as described in this study would still be evident.

5.4.2 Interactions between spinal and supra-spinal regions are likely important in acquisition of persistent high-stepping

A number of studies have shown that spinal circuits alone are able to learn new locomotor behaviour. A clear demonstration of this is that the spinalized cats can recover stepping after training (Barbeau and Rossignol 1987). The spinal cord can also adjust walking to the external environment , for example by increasing flexion to avoid obstacles in the environment (Edgerton et al. 2004, Lou and Bloedel 1988a)or decreasing stance duration to accommodate a force field applied to the leg(Timoszyk et al. 2002). It is also clear that some forms of locomotor adaptation require supra-spinal areas. Cats that have been spinalized generally do not show the same adaptation to ankle extensor loss as intact cats (Bouyer et al. 2001) and intact cats that have been repeatedly subjected to loss of ground contact show an anticipatory adaptation that is not present in spinalized cats (Hiebert et al. 1994).

We have shown that the cortex is necessary for the development of the persistent hyperflexion described in this study (see Chapter 5). It is also likely that the cerebellum is necessary, as earlier studies implicate the cerebellum in other locomotor adaptations, such as adapting to changing treadmill speeds (Rand et al. 1998, Yanagihara and Kondo 1996), rotating disks (Earhart et al. 2002) and environmental perturbations (Lou and Bloedel 1986, Lou and Bloedel 1988b). An intriguing role for the interactions between the cortex and the cerebellum can be proposed based on evidence from another form of

plasticity in the locomotor system which depends on the cortex, conditioning of the H-reflex (Wolpaw and Tennissen 2001). Recently, it has been shown that the dentate and interpositus nuclei of the cerebellum are essential for down-conditioning of the H-reflex in rats (Chen and Wolpaw 2005). Based on previous studies which showed that the tracts that contain descending outputs from these nuclei are not essential for the conditioning of the H-reflex (Chen and Wolpaw 1997, Chen et al. 2002), the authors conclude the important output from cerebellar nuclei is the ascending projection to the cortex. Although it is not yet clear whether the cerebellar output is simply necessary for cortical activity which leads to plasticity, or whether the cerebellum is itself the source of plasticity, it seems likely that cortical-cerebellar interactions are involved in the persistent hyperflexion we describe. Additional inputs certainly come from visual, auditory and integrative regions of the cortex as an important feature of the persistent hyperflexion is that its expression is dependant on context.

5.4.3 Expression and maintenance of persistent high-stepping likely involves descending signals from supraspinal areas and possible alterations in spinal circuits

The areas of the nervous system that are involved in the expression and maintenance of locomotor adaptation are not clear. Most of the significant changes in reflex strength that follow muscle denervation in cats persist following spinalization, suggesting they involve changes in spinal circuits (Whelan and Pearson 1997). Carrier *et al.* (1997) made a similar conclusion, observing that an animal that had recovered from the denervation of ankle flexors and was subsequently spinalized displayed an unusual asymmetrical pattern of gait. Correct expression of this reorganization required intact

supraspinal areas, which indicated the adaptation to the neurectomy required diffuse areas to be expressed. Pearson *et al.* (1999) also suggested a role for both central and peripheral involvement in locomotor adaptation, as recovery from the loss of ankle extensors included both enhanced reflexes and altered central outputs. The expression of down-conditioned H-reflexes is also thought to involve multiple sites, including the montoneuron membrane, mono-and disynaptic connections to the motoneuron, as well as synapses on the contralateral (unconditioned) side of the body (Wolpaw and Tennissen 2001). The persistent hyperflexion that we describe is also likely to involve diffuse regions of the locomotor system, particularly because this adaptation is dependant on context (Figure 4.6). This implies that cortical regions must be involved in recognizing the visual or auditory cues associated with this context, and subsequently lead to the initiation of hyperflexion. In one animal, the speed of walking was also a factor in the expression of hyperflexion (Figure 4.7). We cannot say whether proprioceptive feedback about the speed of walking, or a central signal related to walking speed (Jordan 1998, Whelan 1996) is responsible for this specificity.

While it is likely that associative cortices are crucial for recognizing the contextual cues, the motor cortex is a strong candidate to initiate and maintain the hyperflexion once these cues are recognized. Lesion studies (Drew et al. 1996, Drew et al. 2002) as well as single-unit recordings (Widajewicz et al. 1994) show activity of the motor cortex to be important for increasing flexion of the hindlimb to negotiate obstacles. Furthermore, recent studies have shown that microstimulation of the motor cortex enhances ST activity and increases joint flexion (Bretzner & Drew 2005a), as well as increases the strength of cutaneous reflexes (Bretzner & Drew 2005b). These are all

features of the hyperflexion behavior described in Chapter 4. One intriguing finding from the current study was that the denervation of the knee flexors PB/ST eliminates the long-lasting nature of the hyperflexion, although short-term increases in step height remain (Figure 5.8). We believe that these short-term increases are due to compensatory activity in LG and MG muscles, which cross both the knee and the ankle joint (Tachibana et al. 2006). However, the absence of long-lasting hyperflexion is consistent with the hypothesis that activity from the motor cortex aimed at increasing activity in knee flexors is an important component of the persistent, context-dependant adaptation. Lesioning (Drew et al. 2002) or temporary inactivation (Beloozerova & Sirota 1993) of the motor cortex in animals that had previously developed persistent hyperflexion would be one useful technique to evaluate this hypothesis. Single-unit recordings of the motor cortex before, during and after persistent hyperflexion (Widajewicz et al. 1994) would also help to confirm or deny this hypothesis.

We can only speculate whether spinal circuits are also the sites of adaptive changes following persistent perturbations during walking. We know that they are not sufficient for long-term adaptation of stepping (Figure 5.9) but we do not know their role in the intact locomotor system. In a preliminary attempt to answer this question, we performed a decerebration on one animal that displayed a persistent hyperflexion after repeated perturbed steps in the intact state. If, in the decerebrate state, the stepping of the leg that had previously hyperflexed was markedly different from the contralateral leg, we could conclude that the hyperflexion involved changes in the spinal circuits controlling stepping. This is a similar approach to that taken by (Carrier et al. 1997). We saw no relevant differences in this animal, although further study is necessary before we

conclusively say whether changes at the spinal level are an important part of persistent hyperflexion.

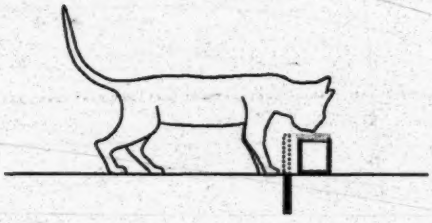
5.5 Conclusion

In this chapter, I have presented two potential roles for the cortex during modifications of the locomotor output. The first is a role during the acquisition as well as the maintenance of long-lasting, context dependent adaptive responses to environmental perturbations. During the acquisition phase, the integration of contextual information from auditory and visual cortices with ascending signals from the cerebellum, possibly in the parietal cortex, would establish the context-dependent adaptive response. Later, in the appropriate context, the motor cortex would be activated to express this adaptive response. Further studies, such as temporary inactivation of the cerebellum during repeated perturbed steps, will provide information to support or refute this hypothesis.

The second role for the cortex in adaptive modifications of the locomotor system involves the modification of the stepping of the hindlegs to avoid obstacles. Recently, we have shown that this process is guided by a memory that is very precise and long-lasting, and is likely to be established in part by the movement of the forelegs. Cortical recordings from the parietal cortex during this task will provide important insight the role of sensory input and motor efference in this process. Understanding how inputs related to the outside world are integrated with ongoing movements of the body will have important implications for locomotion as well as other natural movements in humans and animals.

5.6 Figures

A



B

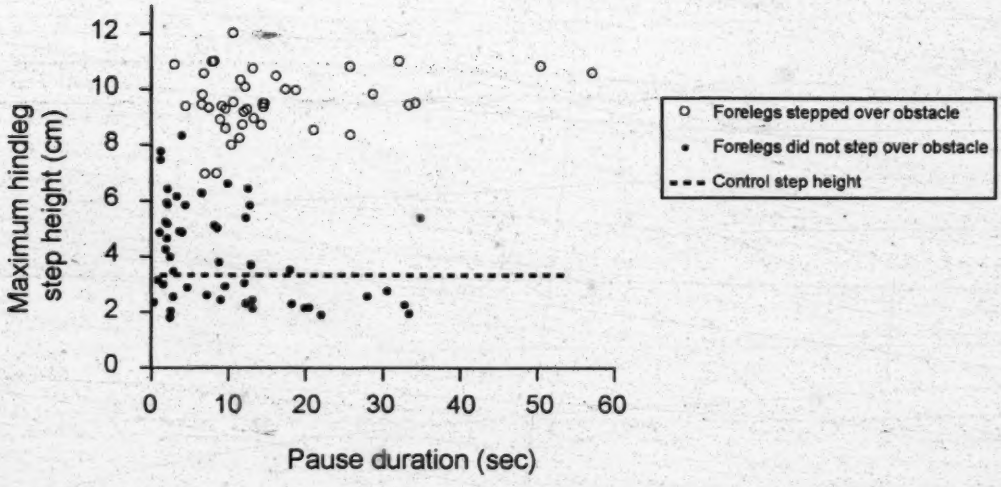


Figure 5.1

The step increase following a pause over an obstacle depends on the movement of the forelegs over the obstacle. A) Cats were paused after the head, but neither set of legs, had passed over an obstacle. B) There was an increase in hindleg step height for only very short delays when the forelegs did not step over the obstacle. Furthermore, this increase in step height was smaller than when the forelegs stepped over the obstacle, and often there was no increase in step height compared to control steps.

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